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Paul Timothy Pollak

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AMIODARONE TOXICITY: RELATIONSHIPS TO CUMULATIVE DOSE AND SERUM DRUG CONCENTRATIONS WITH PRELIMINARY OBSERVATIONS OF CHANGES IN SUPEROXIDE DISMUTASE ACTIVITY

by

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ABSTRACT

Amiodarone, a cardiac antiarrhythmic agent new to North America, provides greater efficacy, more convenient dosing, and produces fewer acute adverse effects than most other antiarrhythmic agents. Toxic effects do occur during chronic therapy, of which pulmonary fibrosis is the most serious. Relationships between adverse effects, serum drug concentrations and administered dose are not yet well understood. This information would be of benefit in treating patients. Lamellar inclusions observed in the lung with amiodarone pulmonary toxicity resemble those from damage by superoxide free radicals in paraquat-induced pulmonary fibrosis. Observation of superoxide dismutase (SOD) activity in patients receiving amiodarone was considered a possible source of preliminary evidence that amiodarone alters free radical metabolism. A liquid chromatographic method was developed for measuring serum amiodarone and desethylamiodarone (DEA) concentrations and access to an assay of SOD activity was attained. Thirty-one patients taking amiodarone completed a one year prospective study to evaluate relationships between serum drug concentrations, drug effects, drug dose and erythrocyte SOD activity. Effects observed were: 6 cases of subclinical pulmonary toxicity with sustained falls in pulmonary diffusion capacity, one of which progressed to overt pulmonary toxicity after completing the study; 8 cases of enzyme elevations diagnostic of mild hepatitis, and 17 cases of abnormalities in thyroid biochemistry. Concentration-response curves for the study population revealed correlations between log serum DEA concentrations, QT interval, corneal microdeposits, serum transaminases, and serum creatinine. Elevations of serum cholesterol and triglycerides occurred. Mean DEA concentrations were closely related to mean amiodarone concentrations and to accumulated dose of amiodarone during the

one year study. Elimination half-lives of amiodarone and DEA were estimated to be 56 and 129 days respectively. SOD activity declined in the group of patients with subclinical pulmonary toxicity and fell 27% from baseline in the patient who later developed overt pulmonary toxicity.

Results suggest that relationships exist between serum drug concentrations, accumulated dose of amiodarone, and several drug effects. Records of QT interval, corneal microdeposits and total accumulated dose may be useful aids in the clinical assessment of the adequacy of therapy and risk for adverse effects and may complement therapeutic drug monitoring. Elimination constants for amiodarone and DEA may be estimated in individual patients without stopping therapy by serial measurement of drug concentrations during the extended accumulation phase. Apparent differences in patient susceptibility to the pulmonary, hepatic and thyroid effects of amiodarone suggest that investigation of genetic differences in metabolism may prove useful. Changes observed in SOD activity in patients developing pulmonary toxicity indicate a need for future investigation into the possible contribution of free radicals to amiodarone toxicity.

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DEDICATION

To my parents.

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GENERAL INTRODUCTION

Among the antiarrhythmic agents currently approved for clinical use in North America, amiodarone is one of the most interesting and controversial. Although its markedly different pharmacokinetic profile and unusual mechanism of action are not fully understood, amiodarone has taken a place at the forefront of antiarrhythmic therapy because of its remarkably high efficacy in treating potentially fatal ventricular arrhythmias resistant to other agents. Its low incidence of acute adverse effects and convenient once-a-day dosing make amiodarone highly acceptable to patients. Unfortunately, toxicity during chronic therapy complicates the use of this agent. Slow elimination of the drug after withdrawal of amiodarone makes the treatment of adverse effects difficult. It is hoped that a better understanding of the mechanisms of toxicity and improved methods for identifying the patients at risk can make the use of amiodarone safer and therefore available to more patients who might benefit from such an effective antiarrhythmic agent.

The results of a study undertaken to investigate the relationship between the effects of amiodarone and serum concentrations of the drug and its major metabolite are described in the following chapters. A secondary goal of the study was to look for preliminary evidence that free radical metabolism might be involved in the mechanism of toxicity with a view to the possibility of using a marker of free radical activity to identify patients at risk for developing toxicity.

CHAPTER 1 - CURRENT KNOWLEDGE

1.1 THE DEVELOPMENT OF AMIODARONE AS AN ANTIARRHYTHMIC

Amiodarone is one of a series of benzofuran derivatives produced in 1961 by Labaz Laboratories of Belgium in a search for a new coronary vasodilator (Charlier et al 1962). Its precursor, benzarone (Fig. 1-1), was synthesised from the benzofuran moiety contained in the coronary dilator, khellin (Singh 1983). Clinical development of benziodarone (Fig. 1-2), the first iodinated derivative of benzarone, was abandoned because of hepatotoxicity in man (Cahal 1964).

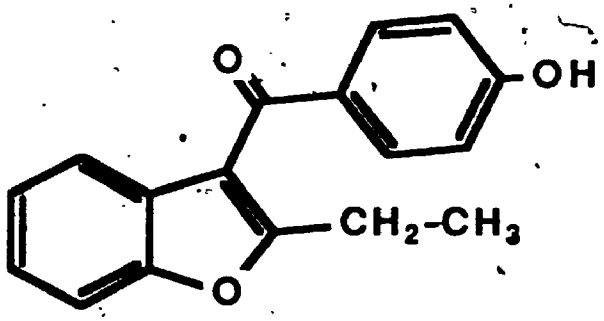

Figure 1-1. Benzarone.

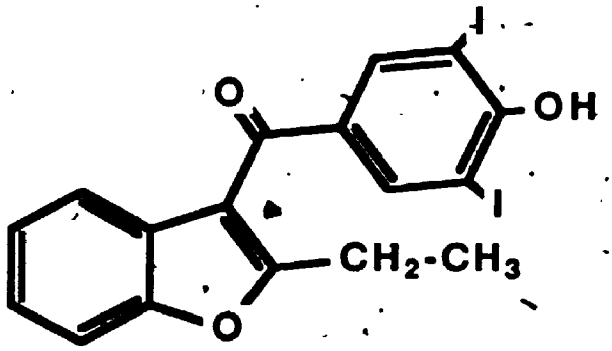

Figure 1-2. Benziodarone.

Amiodarone (Fig. 1-3) is a more potent iodinated derivative of benzarone, first introduced in Europe as an antianginal agent (Vastesaeger et al 1967). The hydrochloride salt of the drug is used in the standard tablet formulation. The doubly iodinated aromatic ring structure of amiodarone bears some resemblance to the structure of thyroxine (Fig. 1-4). Two iodine atoms per molecule of amiodarone hydrochloride account for 37.3% of the 681.8 Dalton molecular weight. It is a white crystalline powder very poorly soluble in water, soluble in alcohol and freely soluble in chloroform. Instability in saline and poor solubility in aqueous solutions cause extreme difficulty in conducting *in vitro* experiments with amiodarone (Charlier et al 1968).

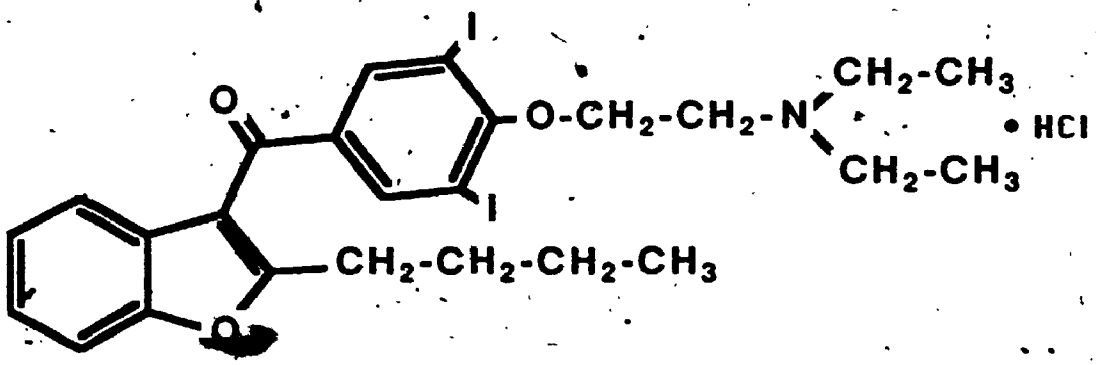

Figure 1-3. Amiodarone hydrochloride.

2-butyl-3-benzofuranyl, 4-(2-(diethylamino)-ethoxy)-3, 5-diiodophenyl ketone, HCl.

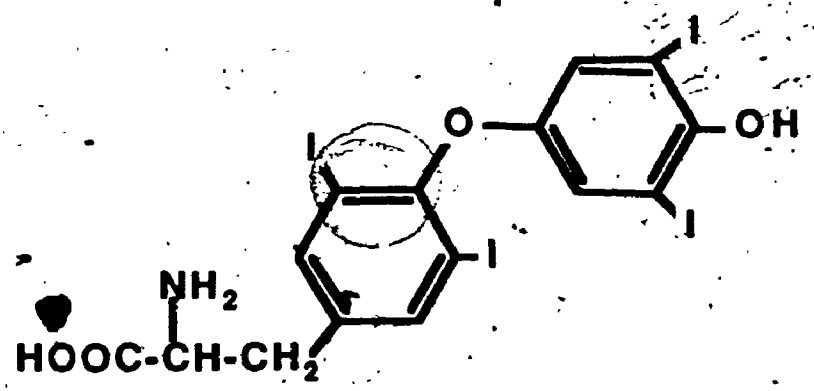

Figure 1-4. Thyroxine.

The compound L8040 (Fig. 1-5) is structurally similar to amiodarone providing a useful internal standard for high performance liquid chromatographic (HPLC) assays. L8040 co-extracts with amiodarone during sample preparation yet has enough structural differences to shift its chromatographic peak away from that of amiodarone (Latini et al 1984a).

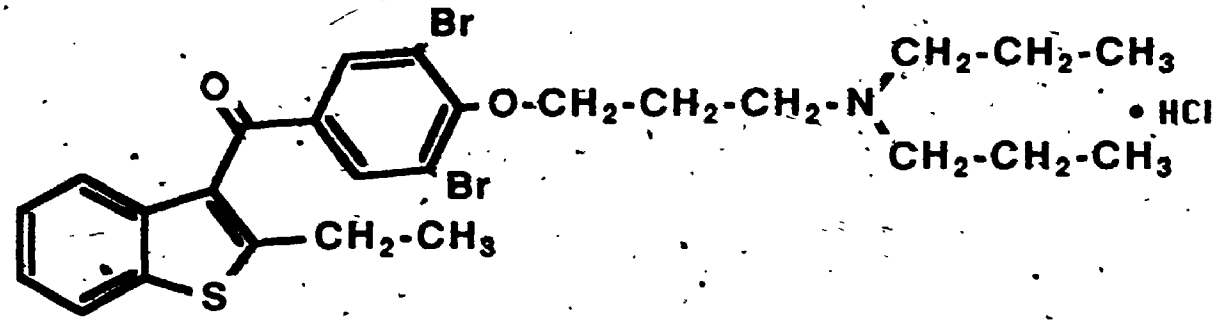

Figure 1-5. L8040-HCl (Chromatographic Internal standard).

Further studies of amiodarone at the Labaz Laboratories led to the discovery of its antiarrhythmic potential (Charlier et al 1969, Charlier 1970). Singh and Vaughan Williams (1970) explored the similarities between the electrophysiological effects of amiodarone and those of the hypothyroid state. Van Schepdael and Solvay (1970) were the first to utilize amiodarone as an antiarrhythmic agent in humans and trials in Europe proved it to be an effective agent for the control of both supraventricular and ventricular arrhythmias (Soussi and Colonna 1974, Benaim et al 1976). Rosenbaum et al (1974 and 1976) conducted large clinical trials in Argentina and reported the first studies in the North American literature. In the 1980's, this unique antiarrhythmic agent has been subjected to intensive study in North America and was released recently for clinical use in both Canada and the United States.

1.2 CARDIOVASCULAR EFFECTS OF AMIODARONE

Although the electrophysiological profile of amiodarone has been under study since 1970, the exact mechanism of its antiarrhythmic action is still not completely understood. As noted above, the drug is not soluble in aqueous media making tissue superfusion studies difficult. Early studies by Singh and Vaughan Williams, measured action potentials recorded in cardiac tissue from rabbits treated with intraperitoneal amiodarone for various lengths of time. The drug was found to have no effect on the resting potential, maximal rate of depolarization, amplitude of the action potential or conduction velocity. The refractory period of cardiac tissue was prolonged by a lengthening of the action potential duration. This did not reach maximum effect until at least 6 weeks of drug administration. No decrease in thyroid function was found and the effects of the drug could not be reproduced by intraperitoneal injection of iodine equivalent to the amount given as amiodarone. The effects of amiodarone could be prevented by intraperitoneal injection of 5 μg of thyroxine daily. It was concluded that amiodarone produced the same electrophysiological effects as thyroidectomy in rabbits (Singh and Vaughan Williams 1970). The ability of amiodarone to prolong the action potential duration in both atrial and ventricular tissues was thought to be its primary mechanism for preventing arrhythmias and led to its classification as a Type 3 antiarrhythmic (Olsson et al 1973). Pritchard et al (1975) demonstrated that the QTc (QT interval corrected for the influence of heart rate) was prolonged in the patients taking amiodarone.

Recent animal studies have demonstrated depression of myocardial and His-Purkinje conduction due to potent sodium-channel blockade (Mason et al 1984, Yabek et al 1985). This blockade differs from that of other sodium-channel blockers in that only inactivated sodium channels are affected. Thus there is

little effect on conduction in fully repolarized myocardium and consequently amiodarone has less activity during bradycardia than during tachycardia when the sodium channels are more frequently in an inactivated state (Mason et al 1984).

The cardiovascular effects of amiodarone after intravenous administration differ from those after oral administration, but both routes have a beneficial effect on ischemia and reduce infarct size in dog models (DeBoer et al 1982). During intravenous administration hemodynamic effects predominate. The heart rate is slowed while the vasodilatory effects of amiodarone lead to an increase in coronary blood flow and a drop in systemic resistance which decreases the left ventricular work load (Charlier et al 1968). These pharmacological effects result partly from an incomplete and non-competitive α- and β-adrenergic blockade (Charlier et al 1968, Polster and Broekhuysen 1976) and may be enhanced by a weak calcium antagonist activity (Gloor et al 1983). None of these acute effects on receptors appear to be responsible for the antiarrhythmic efficacy of amiodarone. During chronic oral administration several electrophysiological changes become apparent which may contribute to an antiarrhythmic effect. Sinus automaticity is decreased with a slowing of depolarization in the sinoatrial (SA) node (Goupil and Lenfant 1976). The atrioventricular (AV) node and His-Purkinje conduction speed is decreased, the action potential duration in all cardiac tissues is prolonged and the refractory period of these tissues increases (Olsson et al 1973). Conduction is also slowed in the accessory pathways of patients with Wolff-Parkinson-White syndrome (Finerman et al 1982). There is minimal negative inotropic effect during chronic administration in humans, but intravenous injection of 5 mg/ kg in a dog model produces an acute reduction of 30% in contractility (Kobayashi et al 1983). The overall effect

on cardiac function is determined by the balance between corenary blood flow, systemic resistance and negative inotropic effect (Schwartz et al 1983).

1.3 PHARMACOKINETICS OF AMIODARONE

Early studies recognized that the effects from administration of amiodarone to dogs were slow in onset and did not peak for 5 to 7 weeks during therapy while heart rate and blood pressure did not return to normal for several weeks after discontinuing the medication (Charlier et al 1968). The protracted time course observed for the pharmacodynamic effects of this medication has been partly explained since the development of methodologies for measuring amiodarone concentrations. This has allowed the partial documentation of the unusual kinetic profile of this drug including an extremely long elimination half-life (Latini et al 1984b).

Pourbaix et al (1985) found absorption following oral administration of both liquid and tablet forms was slow (peak concentrations 3-7 hours after a single oral dose) and incomplete (39 to 100%). This produced a two-fold intersubject variation in the maximum concentrations of the drug in their patients. They also noted the rapid appearance in the blood of the desethyl metabolite which reached higher concentrations after oral than after intravenous administration. Such evidence for first pass metabolism is supported by a study of drug and metabolite concentrations in portal and hepatic vein blood. Berdeaux et al (1984) used this technique to calculate ratios of portal to hepatic vein blood concentrations (an extraction ratio) of 0.39 ± 0.07 for amiodarone and 0.34 ± 0.03 for desethylamiodarone (DEA) at 4 hours post dosing. The average extraction ratio over the period of absorption is likely lower than these numbers.

The appearance of DEA in the portal vein implies that some metabolism of the drug occurs prior to its reaching the liver. Amiodarone is 96% bound to plasma proteins during circulation (Lalloz et al 1984). Neither the parent drug nor metabolites are dialyzable (Bonati et al 1983 and Harris et al 1983a). Less than 1% is excreted unchanged through the kidneys. The primary route of elimination is by hepatic N-dealkylation of the amino group (Figures 1-6 and 1-7) and excretion via the biliary tract (Latini et al 1984b). High concentrations of unchanged amiodarone are found in the bile, suggesting that there is enterohepatic recirculation (Andreasen et al 1981).

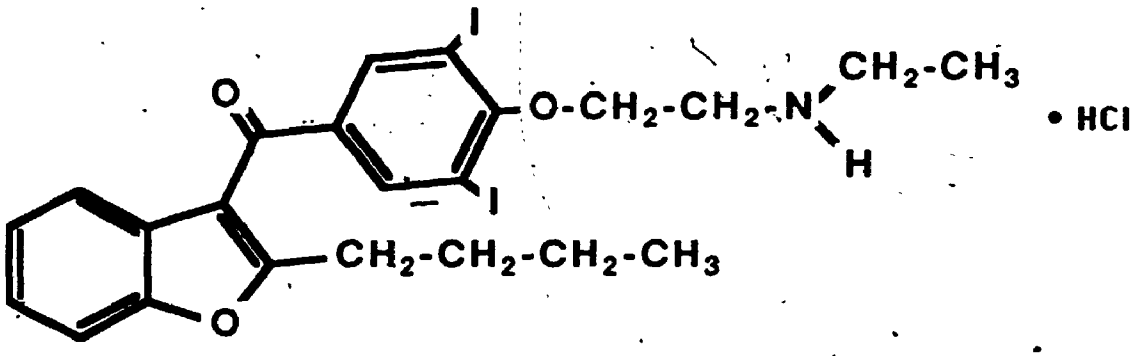

Figure 1-6. Desethylamiodarone.

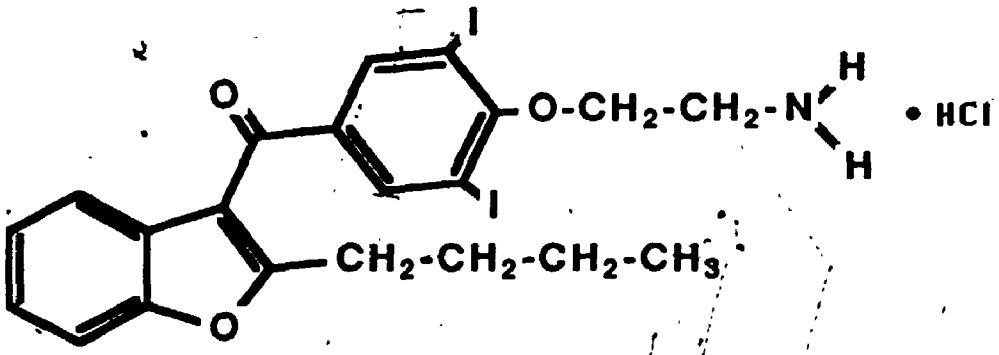

Figure 1-7. Di-desethylamiodarone.

DEA appears in the circulation soon after administration of amiodarone and accumulates to levels from half to twice that of the parent drug (Adams et al 1985). The primary amine metabolite, di-desethylamiodarone (DDEA) has been detected in dogs, but was not detectable in human plasma samples (Latini et al 1984a). Stäubli et al (1983) found that in their patients DEA alone could account for only 20% of the iodine not associated with parent drug or thyroid hormones. They concluded that some of the iodine administered as amiodarone exists as unmeasured metabolites, the most likely candidate being DDEA.

During chronic therapy, extensive accumulation of both amiodarone and DEA occurs in adipose tissues and highly perfused organs such as liver, lung, pancreas, heart and kidney (Maggioni et al 1983). Amiodarone concentrations are higher than those of DEA in plasma and in adipose tissue while the converse is true in most other tissues (Adams et al 1985). Differences in the relative partitioning of amiodarone and DEA found in tissues at postmortem are illustrated in Fig. 1-8 (drawn after Adams et al 1985).

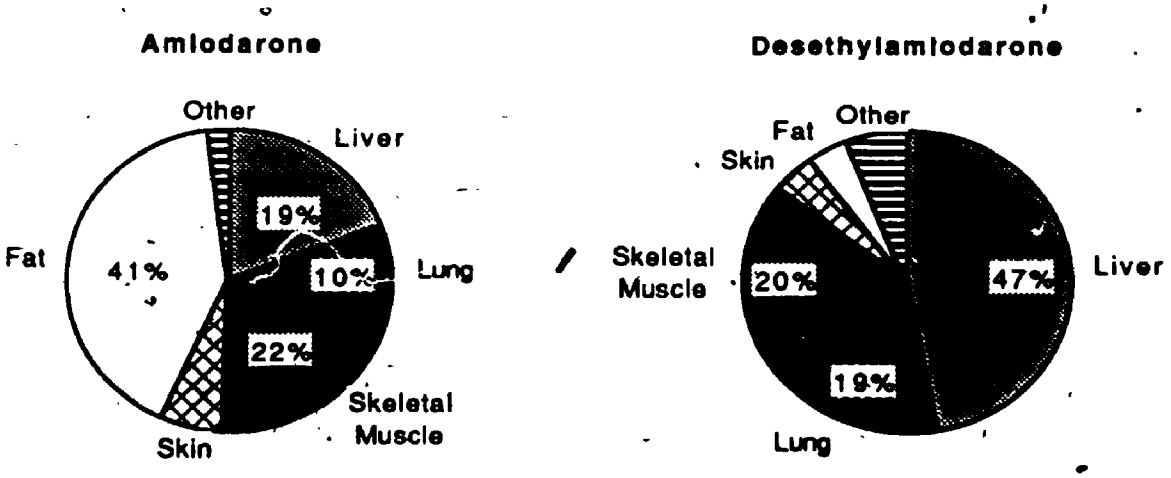

Figure 1-8. Distribution of amiodarone and DEA in tissues.

During chronic administration to dogs, Latini et al (1983) found myocardial/plasma concentration ratios for amiodarone and DEA of 41 ± 26 and 116 ± 68 respectively. Concentrations of amiodarone measured in atrial tissue obtained at surgery in 9 patients on chronic amiodarone therapy correlated with the degree of QTc prolongation (Debbas et al 1984). The extremely high tissue concentrations of amiodarone and DEA found by Holt et al in autopsy material from patients who had received amiodarone are depicted in Fig. 1-9 (drawn after Holt et al 1983). The concentration of DEA to values 4 to 5 times that of amiodarone in the lung and liver deserves note because of the well recognized delayed toxic effects in these organs.

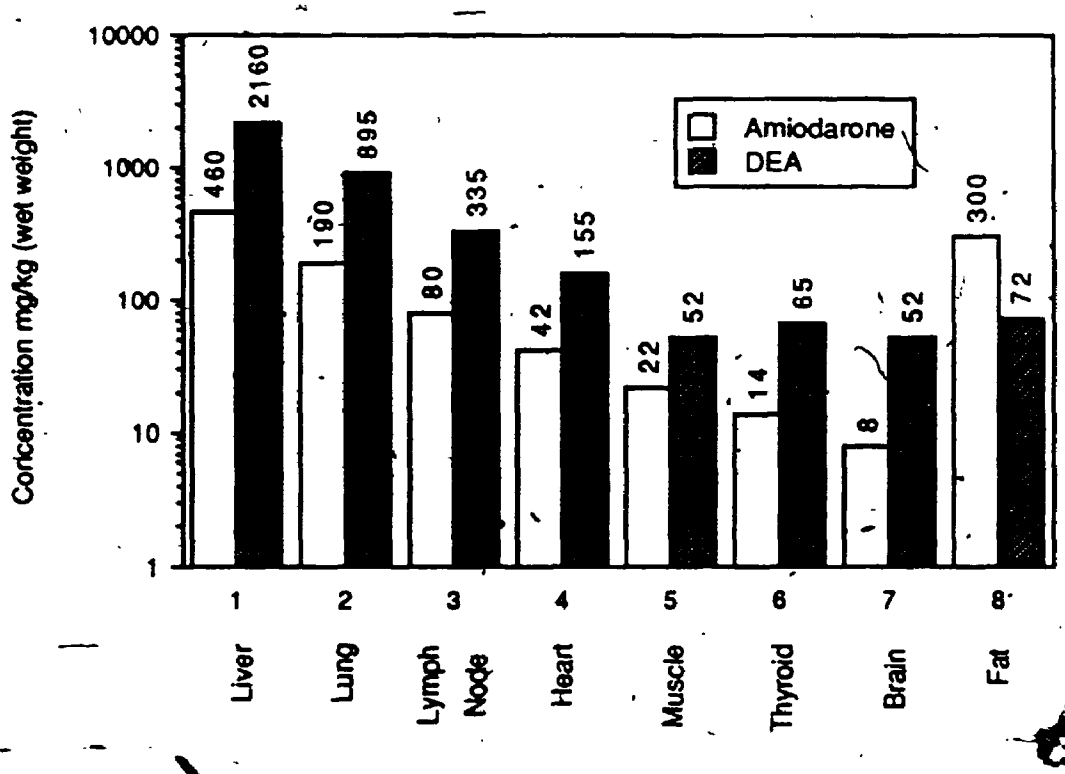

Figure 1-9. Concentrations of amiodarone and DEA in tissues.

Disappointing results with the use of intravenous amiodarone in the acute therapy of arrhythmias have led to speculation that accumulations of the drug or

its metabolite(s) in cardiac tissue are necessary for efficacy. It has been proposed that DEA, which accumulates to higher concentrations than amiodarone, may posses important pharmacological activity (Kannan et al 1982a) as do the N-dealkylated metabolites of other antiarrhythmics such as lidocaine (Kates 1984). Venkatesh et al (1986b) observed that while amiodarone concentrations are high in serum, myocardial tissue and sarcolemmal membranes during acute intravenous administration and decline during chronic maintenance dosing, the opposite pattern is true for DEA. The antiarrhythmic effect of amiodarone is poor during acute administration but increases with chronic therapy (as does the concentration of DEA) which suggests tissue concentration of DEA may be more closely related to electrophysiological activity. Yabek et al (1986) found that DEA produces electrophysiological effects which are qualitatively and quantitatively similar to those of amiodarone in an isolated myocardial superfusion model. Nattel (1986) found that DEA was more potent than amiodarone in producing concentration-related increases in QTc and suggested this was related to a higher partition ratio into myocardial tissue. Venkatesh et al (1986a) demonstrated that administration of DEA to rats produces elevated concentrations of reverse triiodothyronine, a pharmacological activity similar to that of amiodarone. Fraser et al (1984) found that serum DEA concentration was the strongest parameter in a discriminant analysis for predicting a worsening of pulmonary function in their patients.

The extremely long elimination half-life reported for amiodarone (up to 107 days) is probably a consequence of drug accumulation in tissues (Marcus et al 1981, Andreasen et al 1981, Plomp et al 1984, Latini et al 1984b). Biphasic elimination was observed by Holt et al (1983) in 8 patients with an initial 50% decline in concentration during the first 10 days after withdrawal followed by a terminal elimination phase with a mean half-life of 53 ± 24 days. Marchiset et al

(1985) also found terminal elimination half-lives of 52 days for amiodarone and 63 days for DEA in 12 patients withdrawn from therapy.

The concentration of amiodarone in erythrocytes is similar to and linearly related to serum concentrations (Escoubet et al 1986). Debbas et al (1983) showed a linear relationship between plasma and myocardial drug concentrations. Parenchymal tissue concentrations of drug are also in equilibrium with the vascular fluids. High concentrations of amiodarone in cardiac tissue produced by large intravenous loading doses rapidly diffuse back into the central compartment once the initially high serum drug concentrations begin to fall (Gross and Somani 1986, Venkatesh et al 1986b). Somani et al (1985a-b) found that amiodarone and DEA concentrate in leukocytes to levels similar to those measured in several other tissues. Concentrations of drug and metabolite in leukocytes declined more slowly than plasma concentrations when the therapy with amiodarone was stopped. They suggested that leukocyte concentrations are a better indicator of tissue concentrations than are serum concentrations (Somani et al 1986).

There is still no agreement on the utility of measuring serum or plasma amiodarone and DEA concentrations for the prediction of efficacy or toxicity. Low concentrations in a setting of therapeutic failure indicate an inadequate dose of amiodarone, but neither a direct relationship between serum drug concentration and efficacy nor a threshold toxic concentration has yet been established definitively. Average doses of 200 to 600 mg per day produced plasma concentrations of 1.1 to 3.5 mg/L of amiodarone and similar values of DEA in the patients of Holt et al (1983). Based on the serum concentration of amiodarone at the time of recurrence of arrhythmias after the withdrawal of amiodarone, the lowest concentration of amiodarone consistent with efficacy is 0.5 to 1.0 mg/L (Mason 1987). Staubli et al (1983) found that none of their 12 patients

still had arrhythmias when serum concentrations of amiodarone reached 1.5 µmol/L (1.0 mg/L) and pulmonary effusions in one patient resolved when serum amiodarone concentrations fell below 4.0 µmol/L (2.7 mg/L). Rotmensch et al (1984) stratified their patients into 4 groups based on serum amiodarone concentrations. They found a proportional decrease in recurrence of arrhythmias and an increase in adverse effects with increasing concentration across the groups. Elevation of serum hepatic enzymes and incidence of neuromuscular adverse effects was statistically correlated to increasing serum amiodarone concentration, but pulmonary toxicity and thyroid effects were not. Recurrence of arrhythmias occurred in 47% of patients with concentrations less than 1.0 mg/L and in only 14% of patients above this concentration. They stated that the majority of adverse effects occurred with serum amiodarone concentrations above 2.5 mg/L, a value which they chose on the basis of studies by Haffajee et al (1983a). Since this study, serum amiodarone concentrations between 1.0 and 2.5 mg/L have often been quoted as the therapeutic window for amiodarone. Yet, many other reports such as a recent study of 34 young patients by Kannan et al (1987) have failed to show any relationship between dose, plasma drug concentration and effects. Few studies in the literature have been able to demonstrate any continuous concentration-response relationships for the therapeutic or adverse effects of amiodarone, and serum drug concentrations have not been well correlated with dose administered.

Current knowledge of the unusual pharmacokinetics of amiodarone is still important for the effective clinical use of this drug. The long elimination half-life is reflected in both the delayed clearance of the drug from the body and the protracted time to attainment of steady state after the initiation of therapy. Continued electrophysiological and systemic effects must be expected after the cessation of therapy. These effects may persist for several months if steady-

state concentrations were reached prior to cessation (Kaski et al 1981). Augmented dosing during the initiation of therapy is a rational strategy to avoid a lengthy delay in the onset of antiarrhythmic effect (Siddoway et al 1983). Barbieri et al (1986) found atrial tissue concentrations of amiodarone and DEA were higher in biopsies from patients who received therapy for longer than 28 days than in those treated for a shorter period. They suggested the delay in onset of antiarrhythmic activity may be related to the accumulated dose required to saturate the extensive tissue binding sites in the body. Even large intravenous doses of amiodarone are not consistently successful in the therapy of acute arrhythmias (Blandford et al 1982), but the use of initial intravenous doses has produced earlier suppression of arrhythmias (Mostow et al 1984). Electrophysiological (EP) studies with intravenous amiodarone have shown a disappointing lack of prognostic value for chronic amiodarone therapy (Hamer et al 1981, Nademanee et al 1982a). This is consistent with data showing that prolongation of the action potential does not correlate with the drug concentrations produced by acute intravenous administration but instead occurs in conjunction with accumulation of metabolite in myocardial sarcolemma during chronic administration (Venkatesh et al 1986b). Fisher et al (1986) reviewed the problem of predicting efficacy of amiodarone from EP studies done during the initiation of therapy and concluded that non-inducibility of arrhythmias did convey a better prognosis, but inducibility was not prognostic.

1.4 DRUG INTERACTIONS WITH AMIODARONE

Many drugs used in the treatment of cardiac diseases have been found to interact with amiodarone. Most commonly, amiodarone causes the concentration of a coadministered drug to be elevated above the level expected for a

particular dose. Drugs which have been found to have such pharmacokinetic interactions with amiodarone are listed in Table 1-1.

Table 1-1. Drugs which increase in concentration during coadministration of amiodarone

Drug	Citation
Apiridine	Southworth et al 1982
Digoxin	Moysey et al 1981, Nademanee et al 1982b and 1984, Maragno et al 1984, Fenster et al 1985
Flecainide	Shea et al 1986
Phenytoin	Gore et al 1984, McGovern et al 1984
Procainamide	Saal et al 1984
Quinidine	Tartini 1981, Saal et al 1984

After Mason 1987

The mechanisms of these interactions are not fully understood. Digoxin and warfarin were the first drugs for which interactions with amiodarone were documented (Moysey et al 1981, Nademanee et al 1982b, Martinowitz et al 1981, Rees et al 1981, Serlin et al 1981). Renal and nonrenal clearance of digoxin is reduced in the presence of amiodarone, but displacement from tissue and increased bioavailability of digoxin might also contribute to the elevation of its concentration (Nademanee et al 1984, Fenster et al 1985). In contrast amiodarone demonstrates little effect on the kinetics of warfarin, but rather it may directly augment the anticoagulant effect of warfarin by depressing the production of vitamin K-dependent clotting cofactors (Lalloz et al 1984, Neyroz and Bonati 1985). When amiodarone is used in combination with other antiarrhythmics such as quinidine and procainamide, their dosage must be carefully

adjusted because amiodarone inhibits their hepatic clearance (Tartini et al 1982). Quinidine and amiodarone displace each other from serum protein binding sites (Lalloz et al 1984), yet there are no reports of such interactions affecting the concentrations or activity of amiodarone.

Pharmacodynamic interactions with other medications can be expected knowing the β-blocking, calcium-antagonist and electrophysiological effects of amiodarone. Examples include sinus-node depression with propranolol (Derrida et al 1979), hypotension with diltiazem (Lee et al 1985) and proarrhythmia with quinidine (Tartini et al 1982). The danger of sinoatrial arrest or AV nodal block must be kept in mind when contemplating the administration of amiodarone in conjunction with β-blockers or calcium antagonists. The risk of administering amiodarone in the presence of congestive heart failure or in conjunction with negative inotropic agents is not clearly defined but should probably be avoided (Rotmensch et al 1984, Ellenbogen et al 1985).

1.5 EFFICACY OF AMIODARONE IN CLINICAL USE

Therapy with amiodarone has proven highly effective in preventing a large variety of cardiac arrhythmias. The efficacy of amiodarone in the treatment of the tachycardia associated with Wolff-Parkinson-White syndrome was recognized in early studies by Rosenbaum et al (1974). This effect has since been shown to result from rate dependent shortening of the refractory period of the accessory pathway (Brugada and Wellens 1985). Rowland and Krikler (1980) and Ward et al (1980) found that 80-90% of patients with supraventricular arrhythmias unrelated to Wolff-Parkinson-White syndrome responded to amiodarone. Low dose amiodarone was found to prevent both arrhythmias which occur with bradycardia-tachycardia syndrome (Riccioni and Bartolomei 1981),

but Posse and Zuelgaray (1979) recommended the insertion of a pacemaker in conjunction with amiodarone therapy to prevent possible bradycardia. Ventricular tachycardia which is frequently associated with sudden death in young patients with hypertrophic cardiomyopathy was found to be virtually abolished during amiodarone therapy in contrast to verapamil which produced no effect in these patients (McKenna et al 1981).

Until recently the use of amiodarone in North America was restricted to patients with ventricular tachycardia refractory to several other agents. Often these patients had poor general cardiac function which worsened their prognosis. Three major studies of such patients in the early 1980's used augmented dosing for the first 1-4 weeks followed by maintenance doses of 200 to 600 mg/day to treat such patients. Successful therapy was achieved in 50% of 51 patients (Waxman et al 1982), 64% of 196 patients (Heger et al 1983) and in 78% of 96 patients (Nademanee et al 1983). Flaker et al (1985) used a statistical model to show a significant decrease in the number of sustained arrhythmic events during therapy of 17 patients with ventricular arrhythmias refractory to previous therapy. They achieved a success rate of 76%. Smith et al (1986) studied 242 patients of which 156 had supraventricular tachycardias and found that arrhythmias were suppressed in 81% during the first year. Longer follow-up of this high risk group of patients revealed that 56 died and 64 needed to be withdrawn from therapy by 50 months of study. The actuarial probability of remaining successfully treated over 4 years was only 19%. Mason (1987) concluded that amiodarone can be expected to successfully treat 50 to 70% of refractory ventricular tachycardias for one year but that the rate of success will decrease as duration of therapy increases.

1.6 THE TOXICITY OF AMIODARONE IN CLINICAL USE

As the use of amiodarone increased in the last decade, the number and severity of reported adverse effects has burgeoned. They are summarized in Table 1-2. Major adverse effects occur mainly in the liver, lung and thyroid. Other effects are seen in the neuroepidermal and gastrointestinal systems.

1.6.1 Cardiac effects

The incidence of serious cardiovascular adverse effects related to amiodarone is very low in comparison to other agents and those that occur can be predicted from its pharmacology (Mason 1987). The most clinically obvious effect of amiodarone is a persistent bradycardia which has occasionally caused complications during the administration of anesthesia (Gallagher et al 1981). There are isolated reports of sinus node arrest (McGovern et al 1982) and AV nodal block (Gallagher et al 1981). All antiarrhythmic agents may be proarrhythmic under some circumstances. Induction of both Torsade de Pointes and of ventricular fibrillation by amiodarone have been reported but are very rare complications with this drug (Veglia et al 1978, Keren et al 1982, Westveer et al 1982, McComb et al 1980). Given the QT prolongation with this drug, it may be dangerous to administer amiodarone in the presence of other factors such as hypokalemia or class 1 antiarrhythmics which also prolong the QT interval. The danger of amiodarone therapy worsening congestive heart failure during oral therapy is small. A prospective study of 126 patients receiving long-term therapy with amiodarone failed to show any significant negative inotropic effect using serial measurements of ejection fraction (DePaola et al 1987).

Table 1-2. Reported adverse effects of amiodarone

Reported Adverse Effect	Representative Citation
Neuroepidermal	
Corneal deposits	François 1968, Orlando et al 1984
Impaired vision	Ingram 1983
Photosensitivity	Diffey et al 1984
Pseudocyanosis	Delage et al 1975, Alvinovi et al 1985
Central nervous system	Palakurthy et al 1987
Peripheral neuropathy	Charness et al 1984
Epididymitis	Gasparich et al 1984
Gastrointestinal	
Gastrointestinal intolerance	Harris et al 1983b
Increased transaminases	Harris et al 1983b
Hepatitis	Simon et al 1984
Metabolic	
Hypothyroidism	Jonckheer 1981, Mazonson et al 1984
Hyperthyroidism	Keidar et al 1980, Borowski et al 1985
Elevation of cholesterol	Esterhuysen et al 1983
Elevation of blood glucose	Politi et al 1984
Elevation of triglycerides	Politi et al 1984
Pulmonary	
Pneumonitis	Kudenchuk et al 1984
Exacerbation of asthma	Hunt et al 1984
Pulmonary dysfunction	Veltri and Reid 1985
Cardiac	
Proarrhythmia	Veglia et 1978, McComb et al 1980, Keren et al 1982, Westveer 1982
Sinus bradycardia	Gallagher et al 1981, Kosinski et al 1984
Heart block	Brodine et al 1982, McGovern et al 1982b
Refractoriness to cardioversion	Fogoros 1984
Congestive failure	Waxman et al 1982

After Mason 1987

1.6.2 Ocular effects

The most prevalent extracardiac effect of amiodarone, corneal microdeposits (CMD), was one of the first undesired effects of this drug to be recognized (François 1968). Examination of these microdeposits shows them to be intracytoplasmic lysosomal-like inclusions with concentric membranous lamellae confined to the epithelial basal of the cornea anterior to Bowman's membrane (Kaplan and Cappaert 1982). They are found in 76-100% of patients on chronic amiodarone therapy, but only about 10% of patients experience visual disturbances, usually mild, in the form of visual halos when looking at bright lights at night (D'Amico et al 1981). Verin et al (1972) found that amiodarone accumulates in the lacrimal glands and is excreted in tear fluid. Because the cornea is avascular it is assumed that CMD result from drug in the tear film. This is consistent with the work of Bockhardt et al (1978) who produced CMD in rats with topical application of amiodarone to the cornea. Nielsen et al (1982) could not correlate the concentration of amiodarone in tears with changes in the cornea. Instead they found that total dose and duration of therapy correlate (r=0.61, $p<0.001$) with development of microdeposits. Orlando et al (1984) proposed a 4 category grading system for CMD similar to the system used by Miller (1969). In a prospective study of the development of CMD in 18 patients receiving 200 to 1000 mg of amiodarone per day, they observed an orderly increase in the grade of microdeposits as the duration of therapy increased. One patient taking 1000 mg/day developed CMD in 6 days. By 3 months, 94% had grade I deposits , and by 6 months most patients had grade II deposits. Patients with doses of 400 mg/day and above generally progressed to grade III or IV microdeposits.

1.6.3 Pulmonary effects

Although potentially life-threatening, the most serious complication of amiodarone therapy, pulmonary toxicity, was not observed until 15 years after amiodarone was introduced into clinical use. In 1980, Rotmensch et al described a case of pulmonary infiltrates associated with the institution of amiodarone therapy which resolved after withdrawal of the drug. The next year Heger et al (1981) observed this complication in 3 of 45 patients during a trial of amiodarone in the United States. These patients developed dyspnea, diffuse interstitial infiltrates on chest radiographs and pulmonary fibrosis confirmed by microscopy. In 1982 five groups of investigators reported a total of 17 cases of pulmonary toxicity associated with amiodarone therapy, 5 of whom died (Marchlinski et al, Riley et al, Sobol and Rakita, Waxman et al, Wright and Brackenridge). Most of these cases had a nonproductive cough in addition to the above findings. Since 1982 many other reports have appeared in the literature. Variations on the original findings include cases of amiodarone pulmonary toxicity associated with acute onset of dyspnea (Farmakis et al 1984), necrotizing pneumonitis (Pollak and Sami 1984), pericardial effusion (Clarke et al 1985), appearance of nodules visualized by x-ray tomography (Standertskjöld-Nordenstam 1985), fatal adult respiratory distress syndrome (ARDS) following intravenous iodinated contrast material (Wood et al 1985), and bilateral pulmonary effusions (Stein et al 1987).

Of the 17 cases reported in 1982, the nine treated with corticosteroids recovered while the other 5 died. Zaher et al (1983) reported a case of pulmonary toxicity which recurred during rechallenge with amiodarone but resolved when treated with steroids despite continued use of amiodarone. They suggested the use of long-term low dose steroids for prevention of

pulmonary infiltrates in patients who required continued amiodarone therapy to control their arrhythmia. Quyyumi et al (1983) documented the resolution of pulmonary infiltrates despite the continued use of amiodarone in 2 patients treated with steroids. Three groups have reported cases of recurrent pulmonary toxicity when therapeutic steroids were stopped several months after the patients discontinued amiodarone therapy (Joelson et al 1984, Manresa et al 1984 and Adams et al 1986a) They attributed these recurrences to continued exposure to amiodarone released from extensive tissue stores of the drug. Hence a volume of anecdotal evidence supports the use of steroids in the treatment of amiodarone pulmonary toxicity.

The lack of an accurate denominator (no record of the absolute number of patients exposed to amiodarone exists) makes the estimation of the true overall incidence of amiodarone induced pulmonary toxicity difficult. Dean et al (1987) reported an incidence of 6.4% in 171 patients taking amiodarone. This concurs with a range of 1-10% found in 7 studies reviewed by Mason (1987). Magro et al (1985) reported the highest incidence in the literature at 15.1%. A multicenter follow-up in the western United States also found a high incidence of 13% (Mason et al 1985). In contrast Finnegan and Faragher (1985) followed 193 patients looking specifically for evidence of pulmonary toxicity but were unable to find a single case. Other long-term follow-up trials have not had any cases of pulmonary toxicity (Nademanee et al 1981 and Kaski et al 1981).

It was speculated that the sudden increase in reports of pulmonary toxicity associated with the introduction of amiodarone to North America was related to the use of higher doses than in Europe (Rakita et al 1983). Butland and Millard (1984) suggested that doses of 400 mg per day and higher were related to a higher incidence of pulmonary toxicity, but cases have occurred at doses as low as 200 mg per day (Possi et al 1984). Kudenchuk et al (1984) reported a cor-

relation between pre-existing lung disease and the development of toxicity. Adams et al (1986a) also noted that lung dysfunction pre-existed in many patients who developed pulmonary toxicity. They concluded that the occurrence of toxicity was related to both the dose of drug given and to factors predisposing the patients to pulmonary disease. No relationship between pulmonary toxicity and serum drug concentration has been demonstrated. Rotmensch et al (1984) found that while the incidence of other adverse effects was increased with plasma amiodarone concentrations above 2.5 mg/L, pulmonary toxicity frequently occurred at concentrations well below this level.

In view of the seriousness of this complication it has been recommended that chest roentgenograms and pulmonary function testing be done prior to and periodically during therapy with amiodarone (Quyyumi et al 1983). There is evidence to support the use of serial measurements of pulmonary function including the single breath carbon monoxide diffusing capacity (DCO). Veltri and Reid (1985) showed a temporal relationship between amiodarone administration and deterioration in pulmonary function during the rechallenge of a patient with pulmonary toxicity. Anastasiou-Nana et al (1985) observed that patients with falls in DCO of 10 to 20% tended to remain asymptomatic while those with a greater than 20% fall in DCO had a greater risk of developing clinical and radiological signs of toxicity.

If as suggested by Mason (1987), overt amiodarone pulmonary toxicity is fatal in about 10-20% of cases and the incidence of toxicity is in the order of 6%, the estimated overall risk of fatality from pulmonary complications may be as high as 1% in patients taking amiodarone.

Pathological findings associated with amiodarone-induced lung disease include nonspecific inflammation, interstitial and intra-alveolar fibrosis, hyperplasia of type II pneumocytes, and accumulation of foamy macrophages

(Kennedy et al 1987). The foamy appearance of these macrophages and type II pneumocytes results from lamellar inclusion bodies which can be seen with electron microscopy (Marchlinski et al 1982). These inclusions contain high concentrations of phospholipid and iodine leading to speculation that amiodarone is concentrated in these structures (Adams et al 1986b, Israël-Biet et al 1987). Both biopsies (Kennedy et al 1987) and bronchial washings (Butany et al 1984, Israël-Biet et al 1987) from healthy patients taking amiodarone reveal the same inclusion bodies visualized in the lungs of patients with pulmonary toxicity. Similar inclusions may be seen in most tissues in the body. Although the number of inclusions seen has been correlated with the incidence of adverse effects (Adams et al 1986b) they are probably more an indicator of exposure to amiodarone than they are diagnostic of amiodarone toxicity (Somani et al 1987).

The mechanism of amiodarone induced-pulmonary toxicity has not been determined. In their original report Rotmensch et al (1980) suggested that the occurrence of pulmonary infiltrates in their patient was a "hypersensitivity" reaction similar to that seen in patients taking bleomycin. The putative response of pulmonary toxicity to therapy with steroids would support involvement of the immunological system. Other authors have cited deposition of immunoglobulin and complement (Suarez et al 1983) and inversion of the helper/suppressor T cell ratio in the cells collected from bronchial alveolar lavage (Akoun et al 1984a-b) as evidence for an immunologically mediated mechanism of toxicity. Such findings are, however, uncommon (Kennedy et al 1987).

When Marchlinski et al (1982) first observed the lamellar inclusion bodies typical of amiodarone lung, they remarked on the similarity to inclusions observed in chlorpromazine toxicity. It was hypothesized that these inclusions represented abnormalities in lysosomal membrane turnover caused by the

amphiphilic structure of amiodarone. Rakita et al (1983) supported this theory in their review of the problem for the American Heart Association. Colgan et al (1984) likened the amphiphilic structure of amiodarone to that of chlorphentermine. They suggested that the non-polar aromatic ring of amiodarone facilitates its entry into lysosomes where the polar side-chain associates with phospholipids trapping these complexes inside the lysosome. Costa-Jussà et al (1984) examined the massive proliferation of macrophages found in the lungs of rats given amiodarone. They considered the typical inclusion bodies contained in these macrophages to represent a drug induced lipidosis. The same group (Heath et al 1985) used a rat model to demonstrate a four-fold increase in total lung phospholipid content and an increment in the proportion of phosphatidylcholine. This suggested this resulted from a block in phospholipid catabolism consistent with *in vitro* evidence of phospholipase A_1 and A_2 inhibition. Further experiments have shown that amiodarone is nine-fold more potent than chloroquine in the inhibition of phospholipase A and C in rat alveolar macrophages (Hostetler et al 1986). Dean et al (1987) propose that amiodarone alters phospholipid metabolism causing a direct toxic effect on type II pneumocytes. These cells which produce surfactant may be more susceptible to such damage because of their large phosphatidylcholine content.

As yet the chemical mechanism by which membranes and phospholipid structures are disrupted in tissues exposed to amiodarone has not been identified. Other possible mechanisms producing membrane toxicity, such as free radical peroxidation of phospholipids, have not thus far been explored. Free radicals and their possible relationship to amiodarone toxicity and the appearance of lamellar inclusion bodies are discussed in later sections.

1.6.4 Hepatic effects

Elevation of hepatic enzymes in the serum occurs in at least 15% of patients taking amiodarone (Nademanee et al 1982a, Waxman et al 1982, Harris et al 1983c). Harris et al (1983b) were able to show a correlation between log transaminase concentration and the concentrations of both amiodarone and DEA. Elevations of serum hepatic enzymes to twice the upper limit of normal or greater were reported in 50% and 23% of subjects in two studies (Heger et al 1981, Rotmensch et al 1984). Less commonly clinical hepatitis may occur, leading to a picture similar to alcoholic hepatitis (Simon et al 1984), inflammation and fibrosis (Rigas et al 1986), or micronodular cirrhosis (Shepherd et al 1987). All reported cases have characteristic lysosomal lamellar inclusion (myeloid) bodies. As with pulmonary toxicity, many authors postulate that these structures represent a drug-induced phospholipidosis caused by the amphiphilic structure of the amiodarone (Poucell et al 1984). Goldman et al (1987) found a ten-fold increase in hepatic iodine content in amiodarone treated patients which increased the radiographic density of the liver. This amiodarone associated iodine is localized in the lamellar inclusion bodies and Shepherd et al (1987) speculate this indicates that amiodarone and its iodinated metabolites are trapped within these lysosomes.

1.6.5 Metabolic effects

There are two published reports concerning the effects of amiodarone on lipid metabolism in animals. Kannan et al (1982b) found elevations of triglycerides and total cholesterol in rabbits which were associated with increased concentrations of very low density lipoproteins (VLDL). In contrast, a recent

report found a decrease in triglyceride concentrations and no change in total cholesterol in amiodarone treated rats (Kasim et al 1987). However, data from internal drug company toxicity studies shows elevation of cholesterol in mini-pigs, Fischer rats, beagle dogs, and Dutch rabbits (Cordarone® extended product monograph, Ayerst Laboratories, Montreal, Canada). This would suggest that metabolic alterations in animals are a genuine effect of amiodarone.

Isolated reports of abnormalities in humans have also appeared. Esterhuysen et al (1983) reported an elevation of cholesterol concentration in a patient taking amiodarone for one month which fell 8 weeks after withdrawal from the drug. Politi et al (1984) suggested that amiodarone alters triglyceride and glucose concentrations in some patients. Inspection of their data also shows a rise in total cholesterol in all three of their patients with a fall to initial levels after withdrawal of amiodarone. Pollak and Sami (1984) reported hyperglycemia which resolved 12 weeks after the withdrawal of amiodarone. Thus amiodarone appears to affect carbohydrate and lipid metabolism in experimental animal models and possibly as well in humans.

1.6.6 Thyroid effects

Effects on the thyroid are observed commonly, but their etiology is poorly understood (Borowski et al 1985). Both hyper- and hypothyroidism occur, suggesting differences in patient susceptibility to the effects of amiodarone (Jonckheer et al 1973, Pritchard et al 1985, Burger et al 1976, Jonckheer 1981). Amiodarone is a potent inhibitor of the deiodination of thyroxine (T4) to triiodothyronine (T3) (Aanderud et al 1984, Kannan et al 1984). Meese et al (1985) showed that amiodarone diverts the metabolism of T4 from the production of T3 to the production of the inner ring monodeiodinated form, reverse T3

(rT3). This results in a small transient decrease in the T3 concentration with a concomitant rise in rT3 (Jonckheer et al 1978, Amico et al 1984). A temporary increase in T4 production and a decrease in metabolic clearance of thyroid hormones occurs in response to the decrease in conversion to T3 (Lambert et al 1982). Typically a steady rise in serum T4 concentrations occurs over the first month of therapy with little change in binding globulins (Burger et al 1976). Therefore elevations seen in the FTI of these patients mainly reflect rises in T4. Elevations in thyroid stimulating hormone (TSH) during the first two to three months of therapy (Melmed et al 1981) may be caused by amiodarone binding to nuclear receptors for thyroid hormones in the pituitary thereby blocking the feedback inhibition by T3 (Franklyn et al 1985). Changes in T4, T3 and TSH concentrations during amiodarone therapy have been confirmed in large studies (Singh and Nademanee 1983, Nademanee et al 1986, Mechlis et al 1987).

The resemblance between the cardiac effects of amiodarone therapy and those of thyroidectomy (Singh and Vaughan Williams 1970) has led to speculation that a selective blockade of cardiac T3 receptors is involved in the mechanism of action of amiodarone (Singh 1983). Nademanee et al (1982c) postulated that rT3 concentrations not only reflect the degree of competition between amiodarone and thyroid hormones for receptors but are predictive of both the efficacy and toxicity of amiodarone. Latham et al (1987) suggest that cardiac intranuclear thyroid receptors may be partially saturated at the tissue concentrations of DEA attained during chronic amiodarone therapy. They demonstrated that DEA has affinity for solubilized nuclear thyroid hormone receptors in bovine heart with 50% binding occurring at 10^{-4} M (70 mg/L), but this concentrations this high, non-specific binding to the receptor is difficult to exclude. Speculation that similarity in the molecular structures of amiodarone and T4 (Figures 1-3 and 1-4) produces interference with thyroid regulation should be

viewed with prudence because steric hindrances at the link between the benzene rings of amiodarone prevent it from assuming the biplanar configuration of T4. This may account for the lack of binding of amiodarone and DEA to thyroxine binding globulins (Lalloz et al 1984). Data from Lindenmeyer et al (1984) and Meese et al (1985) showing that iopanoic acid which produces changes in thyroid metabolism identical to those of amiodarone does not have any chronotropic or antiarrhythmic effect, do not support a simple interference with thyroid metabolism as the mechanism of action for amiodarone.

Others have proposed that changes in thyroid function result from the iodine load provided by amiodarone (Burger et al 1976, Martino et al 1984). Each 200 mg tablet of amiodarone contains 75 mg of iodine from which approximately 6 to 9 mg of free iodine is metabolically cleaved. In contrast, the daily intake of the average North American diet is only 1 mg. Ingesting amiodarone elevates the iodine content of the thyroid gland as demonstrated by a decreased uptake of radioiodine (Broekhuysen et al, 1969). Leger et al (1983) found that in patients taking amiodarone, those developing hyperthyroidism had a thyroid iodine content twice that of their euthyroid counterparts. A group of patients given inorganic iodine equivalent to that ingested as amiodarone by a treatment group developed effects contrary to those of amiodarone. Instead they demonstrated a slight lowering of T4 concentrations, making iodine load unlikely to be the sole mechanism of action of amiodarone on the thyroid (Burger et al 1976).

Amiodarone induced alterations in thyroid indices return to normal only several weeks after withdrawal of the drug (Singh and Nademanee 1983). Despite the effects of amiodarone on thyroid hormones, clinical thyroid disease is not common in patients taking this drug (Harris et al 1983b). Many patients who develop symptoms have had pre-existing thyroid disease (Vonckheer

1978). Abnormalities in thyroid hormone concentrations are common in patients taking amiodarone but thyrotoxicosis and hypothyroidism are rare. The criteria for abnormalities of thyroid hormones may need to be modified for this population of patients in order to avoid the overdiagnosis of thyroid disease in them (Nademanee et al 1986).

1.6.7 Other adverse effects

While nonspecific dermatoses occur in about 1% of patients taking amiodarone, photosensitivity occurs in virtually all (Walter et al 1984). Alteration of amiodarone by exposure to long wave ultraviolet light (maximum effect 360 nm) increases skin sensitivity to sunlight. Protection from these wavelengths is afforded only by special sunscreens such as 10% dioxybenzone (Diffey et al 1984). A delay of several weeks in the onset of photosensitivity after starting amiodarone therapy suggests that metabolites accumulating in the skin may be the responsible agents (Ljunggren and Bjellerup 1986). The suggestion by Kaufmann (1984) that pyridoxine supplementation would reduce phototoxicity by preventing possible suppression of melanin formation proved to be ineffective in a controlled study of 46 patients (Murlow et al 1985).

In about 2-3% of patients dark pigmentation accumulates in areas exposed to the sun producing a pseudocyanotic appearance (Delage et al 1975). The pigment in the skin has been established to be lipofuscin (Delage et al 1975, Miller and McDonald 1984, Alvinovi et al 1985) rather than melanin which accumulates in other drug-induced hyperpigmentation such as seen with chloroquine (Walter et al 1984). The ultrastructural appearance of lipofuscin is that of lamellar inclusion bodies (Delage et al 1975). Pigment deposition in the skin is accelerated by exposure to sunlight. Miller and McDonald (1984)

suggest that ultraviolet light activates amiodarone to a free radical state capable of oxidizing lipids to form lipofuscin.

There is a wide variation (3.2% to 74%) in the reported incidence of neurologic adverse effects from amiodarone (Palakurthy et al 1987). Both Palakurthy et al (1987) and Charness et al (1984) found tremors in approximately half of their patients. The former group observed a 10% incidence of peripheral neuropathy in addition to central nervous system effects including Parkinsonian-like dyskinesia, encephalopathy and transient brainstem dysfunction. Colebunders et al (1981) reported the development of a cerebellar syndrome during amiodarone therapy. Central effects exhibited earlier onset and resolved more quickly after withdrawal of amiodarone than did peripheral neuropathy (Palakurthy et al 1987). Pellissier et al (1984) could not find a correlation between neuropathy, daily dose, total dose or duration of treatment. Nerve biopsies from affected patients have been found to contain amiodarone concentrations 80 times those of the serum (Fraser 1986) and Schwann cells with typical lamellar inclusion bodies (Meier et al 1979).

Nausea, vomiting, constipation, abnormal sense of taste, and headache may occur in 10-15% of patients during the initiation of therapy (Rosenbaum et al 1976, Rowland et al 1980, Harris et al 1983c). The overall incidence of clinically important adverse effects in one large study of 217 patients was 19%. Approximately 8.3% required withdrawal from the medication because of thyroid abnormalities, pulmonary infiltrates, hepatic dysfunction, and aggravation of arrhythmias (Raeder et al 1985). Table 1-3 summarizes the incidence of the prominent adverse effects observed during 7 major studies of chronic amiodarone therapy with the weighted averages for the 7 studies calculated.

Table 1-3: Incidence of major adverse effects in 7 studies & one multicenter analysis

	Greene et al 1983	Waxman et al 1982	Fogorde et al 1983	Haffajee et al 1983b	Morady et al 1983	McGovern et al 1983	Smith et al 1986	Total pt. in 7 studies	(Multicenter) Mason et al 1985
No. of patients	70	51	96	122	154	80	242	815	1307
Mean follow-up (mo.)	11	9	8	9	14	15	24	-	13
Adverse effect	Percent of patients affected in each study							Weighted ave. of 7 Studies	
Overall	93	55	73	30	51	86	59	60	64
Neurologic effects	74	10	20	19	35	38	17	28	27
GI intolerance	80	-	4	15	8	34	17	20	18
Skin photosensitivity	24	2	12	20	5	9	24	16	12
Blue skin	33	4	4	-	2	4	5	6	5
Impaired vision	7	6	-	3	6	3	13	7	13
Pneumonitis	10	10	7	1	5	5	1	4	13
Congestive heart failure	13	14	0	2	1	0	-	3	11
Bradycardia	5	10	1	3	1	5	2	3	8
Hypothyroidism	4	0	11	1	4	1	3	4	5
Proarrhythmia	0	2	3	0	1	5	2	2	2
Hyperthyroidism	0	2	3	2	2	0	4	2	2
Hepatitis	0	4	1	0	0	1	0	1	-
Amiodarone withdrawal*	19	22	15	9	10	18	26	18	16

* Indicates withdrawal of amiodarone because of adverse effects.

1.7 FREE RADICALS AND DRUG TOXICITY

A free radical molecule possesses a spin-unpaired electron, which is generally highly unstable. This electron avidly reacts with neighbouring molecules often producing a chemical chain reaction (Del Maestro 1980). The presence of free radicals in biological systems not exposed to ionizing radiation was not widely appreciated until superoxide production by xanthine oxidase was demonstrated by McCord and Fridovich in 1968. Recognition that free radicals are intimately involved in physiological processes followed the demonstration that virtually all cells had evolved biological defenses against them (McCord and Fridovich 1969, Fridovich 1978).

Table 1-4 lists a sample of the increasing number of disease states in which abnormal free radical activity has been implicated (Pryor 1976, Del Maestro 1980, Halliwell and Grootveld 1987). Although free radicals may be involved in the development of many pathologic states it should be recognized that they are an inevitable part of normal cellular oxidation-reduction chemistry and not merely the mistakes of a system run astray (Dormandy 1985).

Examples of the unopposed action of free radicals on biological materials such as lipids and proteins can be observed in daily life since free radicals are responsible for the formation of rancid butter and the cracking of aging leather. Failure of cells in a living organism to defend against free radicals leads to the cytotoxic degradation of macromolecules and the generation of harmful activated oxygen species including superoxides and peroxides. All cells living in an oxygenated environment possess systems of intracellular defenses termed free radical scavengers (McCord and Fridovich 1978).

Table 1-4. Disease states with possible free radical involvement

Drug reactions
(e.g. phenytoin hypersensitivity, ethanol toxicity)

Lung
Paraquat toxicity
Bleomycin toxicity
Hyperoxia
Emphysema
Cigarette-smoke effect
Bronchopulmonary dysplasia
Oxidant pollutants (O_3)
Adult respiratory distress syndrome
Mineral dust pneumoconiosis
SO_2 toxicity

Eye
Cataract formation
Degenerative retinal damage
Retinopathy of prematurity
Photic retinopathy

Skin
Solar radiation
Photosensitizers
Porphyria
Thermal injury
Contact dermatitis

Gastrointestinal tract
Halogenated hydrocarbon injury
(e.g. Bromobenzene, CCl_4, halothane)
Endotoxin liver injury
Oral iron poisoning
NSAID-induced lesions
Alloxan induced diabetes

Heart and cardiovascular system
Alcoholic cardiomyopathy
Atherosclerosis
Adriamycin cardiotoxicity
Keshan disease (selenium deficiency)

Kidney
Heavy metal nephrotoxicity
Aminoglycoside nephrotoxicity
Autoimmune nephrotic syndromes

Brain
Hyperbaric oxygen
Neuronal ceroid lipofuscinoses
Neurotoxins
Parkinson's disease
Aluminum overload

Erythrocytes
Phenylhydrazine
Primaquine
Lead poisoning
Protoporphyrin photo-oxidation
Iron overload
Idiopathic hemochromatosis
Thalassemia (multiple transfusions)

Inflammatory diseases
Rheumatoid arthritis
Vasculitis (hepatitis B virus, drugs)
Glomerulonephritis

Radiation injury

Ageing
Disorders of premature ageing

After Halliwell and Grootveld 1987

These defenses take three primary forms, non-enzymatic scavengers present in membranes and cytosol, enzymatic scavengers of oxygen free radicals† and proteins which physically control exposure of metal ion catalytic sites involved in the formation of many free radicals species.

Examples of non-enzymatic scavengers used to pharmacologically manipulate experimental systems include dihydroxybenzoic acid (DHB) and dimethylsulfoxide (DMSO). Compounds found in the cytosol such as ascorbic acid (vitamin C) or in lipid membranes such as β-carotene and α-tocopherol (vitamin E) are important physiologic free radical scavengers. They protect the integrity of cell structures by detoxifying free radicals through nonspecific reactions. Since cells are not capable of directly increasing the concentration of these compounds in response to free radical exposure, these defences are normally limited. Enzymatic scavengers, on the other hand, are proteins synthesized by cells, the production of which can be induced in response to oxidative stress. These enzymes specifically react with activated oxygen species, a major hazard of aerobic metabolism (Del Maestro 1980). An example of response to oxidative stress is induction of the biosynthesis of the scavenging enzyme superoxide dismutase (SOD) in bacteria. Sublethal doses of paraquat induce SOD production under aerobic conditions but not in the absence of oxygen (Fridovich 1978). Cumulative exposure to oxidative stress may also affect the quantity of SOD produced. Gambert et al (1987) have demonstrated a graded increase in the SOD activity of human fibroblasts with increasing age (i.e. accumulating exposure to oxygen).

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† The nomenclature of the three enzymes involved in free radical scavenging as designated by the Enzyme Commission (Barman 1969 and 1974) is as follows: Superoxide Dismutase E.C.1.15.1.1; Catalase E.C. 1.11.1.6; Glutathione Peroxidase E.C.1.11.1.9.

Sources of free radicals are numerous, including endogenous metabolic processes, metabolism of exogenous compounds and ionizing radiation. Activated oxygen species may be produced directly by any of these sources or result from the reaction of other free radicals with oxygen. These species include singlet oxygen, the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the extremely reactive hydroxyl radical ($OH^{\cdot}$). While H_2O_2 and singlet oxygen do not possess spin-unpaired electrons (i.e. are not free radicals), they are intimately involved in free radical generation and are themselves toxic (Petkau 1982).

Superoxide dismutase is the most ubiquitous of the enzymes evolved in response to the hazards of an oxidizing environment. It prevents the accumulation of the $O_2^{\cdot-}$ species by catalyzing the reaction of two $O_2^{\cdot-}$ and two hydrogen ions to from oxygen and hydrogen peroxide (Del Maestro 1980). Catalase detoxifies the hydrogen peroxide produced by the SOD reaction by splitting it to form water and oxygen.

Glutathione peroxidase, a selenium containing enzyme, neutralizes organic hydroperoxides (ROOH) formed by the action of activated oxygen species on molecules such as unsaturated lipids. Liver toxicity produced by xenobiotic metabolites is markedly augmented in selenium deficient animals because of inadequate Glutathione peroxidase activity (Bus et al 1975). This enzyme requires an adequate supply of reduced sulfhydryl groups in the form of reduced glutathione (GSH). These are regenerated from the oxidized form, glutathione disulfide (GSSG), by the flavoprotein, glutathione reductase. The importance of GSH in the detoxification of xenobiotic compounds such as acetaminophen is conspicuous in the situation of an overdose which greatly increases production of the free radical intermediate of acetaminophen (Mitchell et al 1984, Mitchell 1986). The available GSH is consumed in neutralizing this

metabolite and liver necrosis results unless an exogenous source of sulfhydryl reducing equivalents such as N-acetyl-cysteine is administered promptly (Thrush et al 1982).

The most toxic of the oxygen free radicals, the $OH^{\cdot}$ radical, is capable of damaging genetic material and of reacting with virtually any biologic macromolecule (Brawn and Fridovich 1980). Its formation occurs when $O_2^{\cdot -}$ and H_2O_2 react in the presence of a metal catalyst such as iron (Haber and Weiss 1934). Since no enzyme has evolved to dismute the $OH^{\cdot}$ radical, protection from this radical depends chiefly on the maintenance of low concentrations of $O_2^{\cdot -}$ and H_2O_2 and prevention of the Haber-Weiss reaction by sequestering metal catalysts in specialized proteins. These metalloproteins rapidly bind metals such as copper, zinc, and cadmium in blood and tissues and diminish their toxicity. Iron, the most prevalent metal catalyst is protected from exposure to substrates in proteins such as transferrin and other heme proteins (Editorial Lancet 1985). Failure by genetically abnormal hemoglobin to properly sequester metal ions in red blood cells reduces their life span because $O_2^{\cdot -}$ production and the formation of methemoglobin is increased (Carrell et al 1977). Enzymatic defenses against free radicals such as SOD, are found in high concentrations in erythrocytes where abundant oxygen in close proximity to iron increases the risk of $O_2^{\cdot -}$ formation.

Apart from activated oxygen species, the other major source of free radicals in biologic systems are activated metabolites of xenobiotics or free radicals released by enzymes during the metabolic process. Metabolic activation usually takes place in the liver and this organ is often the primary site of toxicity for drugs with free radical intermediates. Poisoning with halogenated organic compounds and from iron overload are two examples of free radical toxicity where liver damage is predominant (Dianzani 1987).

Poisoning with the agricultural herbicide paraquat (methyl viologen) produces a prototypical syndrome of free radical toxicity (Fridovich 1978, Fairshter 1981). The pathological changes produced in the lungs by paraquat toxicity including infiltration of macrophages, increased lipid concentrations (Fletcher and Wyatt 1970), alveolar inflammation, proliferation of pneumocytes and the development of lamellar bodies (Kimbrough 1974), are very similar to the changes described by Marchlinski et al (1982) and Costa-Jussà et al (1984) with amiodarone pulmonary toxicity. Bus et al (1975) were the first to suggest that lipid peroxidation was involved in paraquat toxicity. It has since been shown that paraquat itself does not react directly with cellular components, but that redox cycling of its bipyridil cation catalyzes the production of superoxide in the presence of oxygen. The resulting activated oxygen species produce the liver and lung toxicity of paraquat (Thrush et al 1982). Serum malondialdehyde concentration, a marker for lipid peroxidation, is increased in paraquat toxicity (Yasaka et al 1986). Depleting non-enzymatic scavengers in animal models enhances toxicity, while supplemental SOD has a protective effect against paraquat toxicity in cultures of S. *typhi murium* (Hassan and Moody 1982). The predominant site of toxicity can be shifted from lung to liver by selenium depletion in mice (Cagen and Gibson 1977). Lethal doses of paraquat apparently overwhelm the enzymatic scavenging system and have been shown to depress SOD activity in lung microsomes to below detectable limits in animal models (Montgomery 1977).

Amiodarone hydrochloride itself is not a free radical, but evidence exists that amiodarone or its metabolites can be activated to toxic states by exposure to light. Therefore it may be profitable to look for evidence of alterations in SOD activity which would take place if tissues are exposed to increased attack by free radicals.

1.8 LAMELLAR INCLUSION BODIES

Lamellar inclusion bodies in the tissue of a patient taking amiodarone were first reported by Delage et al in 1975. These inclusion bodies, seen on light and electron microscopy of a skin biopsy from a patient who developed pseudocyanotic pigmentation (blue skin), were identified as lipofuscin. This pigment accumulates with age and is thought to result from the irreversible cross-linking of intracellular membrane lipids which are sequestered in lysosomes (Sekhon and Maxwell 1974). Nutritional deficiencies which leave the body more susceptible to free radical attack lead to the accumulation of lipofuscins in the liver (Dianzani 1987).

Ultrastructural studies have revealed similar inclusions bodies in patients with a whole spectrum of amiodarone toxicity. Meier et al (1979) reported them in Schwann cells of nerves in association with peripheral neuropathy and D'Amico et al (1981) found them in the corneal epithelium of patients with corneal microdeposits. Marchlinski et al (1982) were the first to report them in lung macrophages from patients with pulmonary toxicity and Simon et al (1984) found them in hepatocytes of a patient with severe hepatotoxicity. Somani et al (1986) concluded that these lamellar inclusions occur in most patients receiving amiodarone, with or without toxicity. Others have suggested that increasing density of lamellar inclusions in neutrophils is correlated with a greater incidence of adverse effects (Adams et al 1986b).

The occurrence of these inclusions is accompanied by the disappearance of normal lysosomes (Lüllmann et al 1978) and is associated with the accumulation of intralysosomal iodine (Shepherd et al 1987). The apparent lysosomal origin of lamellar inclusion bodies is central to most of the theories promoted concerning the etiology of amiodarone toxicity. The extended period of drug

exposure which usually transpires before toxicity occurs is consistent with a mechanism involving the accumulation of drug in lysosomes (Gross and Somani 1986) such as seen with chloroquine (Abraham et al 1968, Hostetler et al 1985). Amiodarone has the same diethylaminoalkyl side chain as chloroquine and other agents known to induce phospholipidosis and hepatitis (Goldman et al 1985). Dake et al (1985) report that these structures represent accumulations of exogenous phospholipid in lysosomes and compare them to inclusions seen in experimental phospholipidosis caused by chlorphentermine (Kacew et al 1977). One author has postulated that toxicity occurs when lysosomes are disrupted by accumulations of phospholipids releasing proteolytic enzymes toxic to the cell (Shepherd et al 1987). Since lysosomes contain the apparatus to produce large quantities of activated oxygen species, free radicals would likely play a role in producing toxicity if these organelles were disrupted in such a way. Two situations suggested by the current knowledge of amiodarone could result in an increase in lipid peroxidation and the production of lipofuscin pigments. Amiodarone could disrupt lysosomes because of its amphiphilic structure, causing the discharge of lysosomally produced free radicals, or it could produce free radicals through its metabolism which by cross-linking of the phospholipids of lysosomal membranes would disrupt their activity.

1.9 SUMMARY

From the information presented it can be concluded that:

1 Amiodarone is an effective antiarrhythmic with the potential to prevent many deaths.

2 The extremely long elimination half-life and large tissue accumulations of amiodarone confer on it the advantage of infrequent dosing and prolonged protection from arrhythmias.

3 These same properties may also be related to the occurrence of a variety of adverse effects which increase in incidence the longer the duration of use. The cornea, skin, liver, thyroid and lung are the most common organs to be affected.

4 Amiodarone exhibits a general tendency to inhibit biologic processes. It blocks α and β-receptors and both sodium and calcium channels. It inhibits phospholipases, the metabolism of many drugs, the conversion of T4 to T3 and the production of clotting factors.

5 Some evidence exists that amiodarone alters the metabolism of lipids and carbohydrates.

6 An understanding of the relationship between serum drug concentrations and adverse effects would aid in the safer administration of this useful agent. Questions still remain regarding the nature of dose-concentration relationships and concentration-response curves. No reports of elimination half-lives estimated from the serum concentration accumulation curve prior to steady state have been made. The common observation that appearance of corneal microdeposits correlates with the magnitude and duration of dose of amiodarone might be clarified by careful comparison to serum drug concentrations.

7 Demonstration of the pharmacological activity of DEA and the increasing incidence of toxicity as high concentrations DEA accumulate in tissues warrants careful attention to the relationship of serum DEA to adverse effects.

8 Ultrastructural abnormalities in the lungs and other tissues of patients taking amiodarone have the same appearance as those seen in lungs damaged by the free radical producing agent, paraquat. The toxicity of amiodarone is greatest in the lungs and liver which are the two internal organs most at risk for attack by free radicals and in the skin and cornea, the two organs exposed to ultraviolet light. These facts suggest a mechanism of toxicity involving free radicals as a candidate for investigation. Apart from reports in the photobiology literature, there has been no investigation into the possibility that amiodarone alters free radical metabolism.

Therefore in addition to observing the relationship of drug and metabolite concentrations to toxicity, it would be reasonable to seek preliminary evidence of changes in free radical activity in humans taking amiodarone.

CHAPTER 2 - METHODS

2.1 INTRODUCTION AND HYPOTHESES

Current understanding of the pharmacokinetic behaviour of amiodarone is incomplete. Attempts to correlate serum amiodarone concentrations with adverse effects have shown only weak relationships. There has been no adequate demonstration of the relationship between dosing and serum drug concentrations. Most studies have compared daily dose with serum drug concentrations which were assumed to have reached steady state within 2 to 6 months after starting therapy. Given that the half-life of amiodarone may be as long as 3 months, an approach to the analysis of concentration data which does not require the assumption of steady state might solve some of the problems demonstrating these relationships. Careful documentation of the evolution of drug related effects and analysis of concentration data under the assumption that steady state may not be reached during the period of study may identify relationships which have previously been missed. Evidence in the literature that the N-desethyl metabolite of amiodarone possesses pharmacological activity suggests that a more careful search for relationships between serum desethylamiodarone concentrations and both the therapeutic and adverse effects in amiodarone treated patients would be fruitful. Similarities between the pulmonary pathology occasionally caused by amiodarone and that caused by agents such as paraquat (noted on pg. 38) which produce toxicity through the action of free radicals on membranes raise a question of the possible involvement of free radicals in amiodarone pulmonary toxicity. No investigations have been attempted yet to look for *in vivo* changes in patients taking

amiodarone which might reflect the interference of this drug in the normal physiology of free radicals.

HYPOTHESES: Relationships exist between serum amiodarone concentration and the effects of this drug and between the dose of drug administered and serum drug concentration which can be demonstrated by careful documentation of clinical effects and accumulating serum drug concentrations. Similar relationships should also exist for serum concentrations of the pharmacologically active metabolite, desethylamiodarone. The possibility that free radicals contribute to amiodarone toxicity can be explored in a preliminary fashion through observation of changes in activity of the enzyme, superoxide dismutase.

2.2 PROPOSED LONGITUDINAL EVALUATION OF PHARMACOLOGICAL AND ADVERSE EFFECTS OF AMIODARONE

The two major research objectives were: 1) collection of clinical data on the effects of amiodarone at intervals frequent enough to allow assessment of the time course over which changes in these parameters occur; 2) measurement of amiodarone and desethylamiodarone (DEA) to provide data for pharmacokinetic analysis and determination of concentration-effect relationships. In parallel with these objectives, observation of changes in superoxide dismutase activity in the erythrocytes of patients taking amiodarone would be carried out to look for possible relationships with serum drug concentrations and observed effects.

A clinical trial was proposed in order to fulfill the first objective. The trial was designed to monitor the effects of amiodarone and to collect blood samples for the measurement of serum amiodarone and DEA concentrations and as a preliminary assessment of changes in erythrocyte superoxide dismutase activity

in patients taking amiodarone. To fulfill the second objective, an improved high performance liquid chromatographic method was developed and validated to allow measurement of amiodarone and DEA concentrations on site since no facilities existed locally for measuring these concentrations and previously published methods were not entirely satisfactory. Support was obtained from a local laboratory which had developed a methodology for the analysis of superoxide dismutase activity. Relationships between observed effects, serum drug concentrations and superoxide dismutase activity were to be investigated.

The basis of this study was to identify differences between patients developing toxicity and those who do not. Analysis was to be carried out on the basis that groups of patients developing certain toxicities could be identified from within the study population. Parameters were selected to detect the effects of amiodarone on the heart, cornea, lung, liver and thyroid. Effects on the heart (Singh 1983), cornea (Ingram 1983) and thyroid (Singh and Nademanee 1983) were expected to occur in virtually all subjects and therefore were expected to be poor discriminators for identifying differences between any groups of subjects which were more or less susceptible to toxic effects of amiodarone. In contrast, major toxicity such as occurs in the liver and the lung, develops in only some patients. The mean incidence of pulmonary toxicity (average of reported incidence weighted for number of patients per study) occurring in 573 patients followed in 6 studies reported in 1982 and 1983 was 5.2 %. This is low in comparison to the incidence of other abnormalities. It was clear that providing a sample size large enough to have a reasonable chance of observing at least one subject who would develop pulmonary toxicity was the limiting factor in designing the size of the study.

The probability (P) of observing y subjects with a certain characteristic; (c) in a sample of the size (n) where the sample is selected from a population in

which c occurs with an incidence of P_c, is described by the following binomial equation (Box et al 1978):

$$P(y) = \binom{n}{y} (P_c)^y \cdot (1-P_c)^{n-y}$$

The case y = 0 describes the probability that the characteristic will not be observed in any subject of the selected sample. The equation is simplified for this case because $(P_c)^y = 1$ and $\binom{n}{y} = 1$, with the result:

$$P(0) = (1-P_c)^n$$

taking the log of both sides of this equation produces:

$$\log(P(0)) = n \cdot \log((1-P_c))$$

therefore:

$$n = \frac{\log(P(0))}{\log((1-P_c))}$$

In a sample of size n, the chance of finding at least one subject with the characteristic, c, is 1-P(0). The sample size necessary to have a 0.8 chance of finding at least one subject with pulmonary toxicity, which is predicted to occur in 5.2% of the population taking amiodarone, can be calculated by substituting the values $P(0) = 0.2$ and $P_c = 0.052$ into the above equation for n. The results of this calculation yields a minimum sample size of 30.

The experience at the site selected for the study was that 60 patients were started on amiodarone during the previous year. An enrollment phase lasting 18 months was projected to provide approximately 90 patients as candidates for the study. Making a conservative estimate allowing for one third being unsuitable and one third not wishing to participate, a minimum of 30 patients could be expected to enter the study before termination of the enrollment phase. It was decided that at least 30 patients should be enrolled but it would be practical to follow up to 40 subjects if the opportunity occurred to enroll them. A larger

number of subjects would allow for drop-outs which were to be expected in this relatively high risk population.

The choice of superoxide dismutase for a preliminary investigation into the possible role of free radical in amiodarone toxicity offered advantages over other free radical scavenging enzymes. Superoxide dismutase is important in the protection of cells against oxygen free radicals, it is found in high concentrations in erythrocytes which not only are obtainable with minimal discomfort to the patients but are exposed to amiodarone and metabolites. The assay for SOD activity is well standardized, relatively simple to perform and laboratory facilities for SOD analysis were available locally. No information was available concerning the magnitude or variability of superoxide dismutase activity in a population with cardiac disease. Documentation of such information by this preliminary study was expected to aid the design of future studies.

2.3 Experimental Design and Methods

Subjects were normally to be entered into the study during hospitalization for control of their arrhythmia. The investigator was informed by the cardiologists in the hospital where the study was conducted whenever a decision was made that amiodarone was appropriate for the treatment of a patient with arrhythmia. The patient was then approached with regards to participating in a prospective study of the adverse effects of amiodarone. Each was interviewed regarding his/her willingness to take the medication, feelings toward participating in a research protocol and appropriateness regarding simple exclusion criteria. Informed consent to participate in the study was obtained from patients after the risks, benefits and adverse effects of amiodarone were explained to each subject. Patients were excluded if they had severe pulmonary disease or

life-threatening extracardiac disease which might interfere with the identification of adverse effects. Subjects also had to be capable of travelling to attend clinic for follow-up six times in the period of a year. At the time of patient enrollment, amiodarone was released for the purposes of emergency use. Use of concomitant medications was minimized to simplify the identification of adverse effects.

2.3.1 Clinical follow-up

Clinic visits were scheduled at 1, 2, 3, 6, 9 and 12 months after starting amiodarone therapy. At each visit the patients were asked about any perceived therapeutic or adverse effects of amiodarone therapy, any changes in their life style and any changes in concomitant medications if applicable, including vitamins. Specific questions were asked about the incidence of arrhythmias, visual changes, skin changes, neurological symptoms and symptoms consistent with pulmonary and thyroid disease. Before starting on amiodarone and at each clinic visit, a complete physical examination was carried out. The skin was inspected for signs of increased pigmentation. The cardiovascular and pulmonary systems were carefully assessed. The heart rate and blood pressure were recorded and the QT interval corrected for heart rate (QTc) was noted from an electrocardiogram at each visit. QTc was calculated from the electrocardiograph using the formula QTc equals the QT interval divided by the square root of the R-R interval (Bazett 1920). Lengthening of the QTc was considered a therapeutic effect.

At each visit, examination with a slit lamp ocular microscope was carried out to determine the grade of corneal microdeposits according to the classification of Miller (1969). Grade 1/2 was added to the classification system to denote

the earliest appearance of fine punctate golden-brown opacities without a defined pattern. Grade 1 of Miller's classification was defined as the coalescence of the deposits in a horizontal linear pattern on the inferior cornea, Grade 2 as the extension of arborizing lines from the ends of the horizontal deposits and Grade 3 as an intensification and extension of the arborized pattern across the visual axis of the cornea to form a defined whorl design. The average score for the two eyes was recorded for each visit. Because of the high frequency and benign nature of this adverse effect, its occurrence was not defined as toxicity.

2.3.2 Experimental sample collection

Before starting on amiodarone and at each clinic visit, blood was drawn for the measurement of amiodarone and DEA concentrations and for the assessment of erythrocyte SOD activity. The serum from the clotted blood of a 10 ml collection tube was separated by centrifugation and frozen at -20° C in polyethylene vials for later analysis of drug concentrations by liquid chromatography. Whole blood from a 5 ml heparinized collection tube was prepared by osmotically lysing 100 µL of whole blood in 1 mL of 10 mM potassium phosphate buffer pH 7.4 and storing the aliquot at -80 °C for later analysis of SOD activity.

2.3.3 Clinical laboratory measurements

Clinical laboratories at the hospital were used to monitor physiologic and metabolic parameters which might be altered as a result of taking amiodarone.

2.3.3.1 Pulmonary function

Following the recommendations of Quyyumi et al (1983), pulmonary function was monitored. Arterialized capillary blood gases, lung volumes and single-breath carbon monoxide diffusion capacity (DCO) were measured before starting therapy and at each clinic visit and chest radiograms were performed a three monthly intervals. Previous investigations had demonstrated that one-third of patients taking amiodarone developed more than a 15% reduction in DCO (Greene et al 1982). The CV of repeated measurements of DCO in patients with moderate lung disease has been reported as 5% (Crapo 1980). Therefore a 20% reduction was defined as abnormal for this study. The criteria for pulmonary toxicity were defined in the study design as the occurrence of any one of the following: 1) interstitial disease on chest radiograph; 2) a sustained decrease in single breath carbon monoxide diffusion capacity (DCO) or forced vital capacity (FVC) to less than 80% of baseline values, persisting over at least two consecutive follow-up visits; 3) cough and dyspnea not explained by congestive heart failure or infectious disease persisting over one week. Patients were to stop amiodarone therapy if any two of these criteria were met or if progressive changes were noted on chest radiographs. Radiographs were interpreted by a radiologist on staff at the hospital.

Blood gases were measured on an ABL^4 Acid Base Laboratory machine (Radiometer AS, Emdrupvej 72, DK-2400, Copenhagen NV) with a coefficient of variation (CV) of 0.1-1.0% for PCO_2 and 1.5-1.9% for PO_2. Experienced personnel in the pulmonary function laboratory performed measurements of pulmonary flow volumes and diffusion capacity using a PK Morgan M8 Dry Rolling Seal Pulmonary Function Analyzer with carbon monoxide delivery system and PK Morgan CO analyzer model 403 (PK Morgan Instruments Inc.,

11522 Pagemill Rd., Dallas TX, 75243). Flows were calibrated with an allowable CV of 1.6%. Three repeated measurements of diffusion capacity were required to agree to within 1.5 ml/min/mmHg. Flow volume and diffusion capacity calculations were performed on a Zenith Z-100 microcomputer with Wyvern Software pulmonary function program version 1.01. Hemoglobin for the correction of the calculated diffusion capacity was measured on an Radiometer OSM3 Hemoximeter with a CV of 0.4-0.5% (Radiometer AS, Emdrupvej 72, DK-2400, Copenhagen NV).

2.3.3.2 Serum chemistry (hepatic and renal function)

Parameters used to monitor liver function were serum bilirubin, aspartate transaminase (AST formerly SGOT), alanine transaminase (ALT formerly SGPT), alkaline phosphatase, glucose, cholesterol and triglycerides. The upper limits of normal for the serum hepatic enzyme concentrations provided by the clinical biochemistry laboratory are calculated from measurements in healthy subjects. Values above these normal limits were common in the cardiac patients considered for enrollment in the study. Rather than exclude patients for the minor abnormalities strict criteria for the definition of biochemical hepatotoxicity were defined. Based on the work of Rotmensch et al (1984) which showed variations in serum transaminases as high as twice the upper limit of normal were common, variations in serum hepatic transaminases were not considered diagnostic of toxicity unless they varied to more than twice their baseline values and these variations were corroborated by similar changes in the other hepatic transaminase. The criteria for hepatic toxicity were defined in the study design as either: 1) elevation to twice baseline value of both serum hepatic transaminases, or; 2) jaundice or hepatic tenderness without other

explanation. Patients were to stop amiodarone therapy if the second criterion was met or if a patient with elevated hepatic enzymes was clinically ill. Prior to the initiation of amiodarone therapy and at each follow-up clinic visit, blood was drawn for routine biochemistry, transaminases, glucose, triglycerides and cholesterol. The patients were asked not to eat before attending clinic, but a full 14 hour fast was often not practical as the patients who required afternoon appointments in order to travel to clinic would participate only if a light breakfast were permitted. Parameters used to monitor renal function were serum creatinine and electrolytes (Na^+, K^+, Cl^-, HCO_3^-).

Clinical biochemistry analyses were performed on a sequential multichannel automated analyzer (SMAII System - Generation II, Technicon Instruments Corporation, Tarrytown, New York). Cholesterol and triglycerides were measured enzymatically in the clinical biochemistry laboratory of the hospital using a separate autoanalyzer (Du Pont-aca®, Du Pont Biomedical Products, Wilmington, Delaware *19898*). The coefficients of variation for the various biochemical assays are as follows:

Na^+	1.7%	Cl^-	1.4%
K^+	2.4%	HCO_3^-	2.2-3.7%
Creatinine	1.7%	AST(SGOT)	2.9-14.0%
Cholesterol	3.4%	ALT(SGPT)	2.9-14.0%
Triglycerides	8.0%	AP	2.6-4.8%
Glucose	1.6-8.3%*	Bilirubin	4.3-10.4%

* where two values appear there is a major change in accuracy between elevated and normal physiological concentrations.

2.3.3.3 Thyroid function

The parameters for monitoring thyroid function available for measurement by the clinical laboratory were triiodothyronine concentration (T3), total thyroxine concentration (T4), T3 resin uptake (RT3U) and free thyroxine index (FTI). The FTI, which reflects both total thyroxine (T4) concentration and thyroid hormone binding in the plasma, was chosen as the most sensitive indicator of abnormalities in thyroid hormone chemistry. A chemical abnormality in thyroid function was defined in the study design as the development of an FTI value outside the upper or lower limits of the normal range (above 0.56 units or below 0.26 units). If clinical changes consistent with thyroid disease developed in conjunction with such abnormalities, the patients were to be assessed by an endocrinologist with regards to the need for withdrawal of amiodarone. Before starting on amiodarone and at each clinic visit, blood was drawn for assessment of thyroid function. Measurements of T4 and RT3U, an indicator of the thyroglobulin binding capacity, were made in the clinical thyroid function laboratory utilizing an automated fluorescence polarization immunoassay (TDx Analyzer, Abbott Laboratories Ltd., Diagnostics Division, 7115 Millcreek Dr., Mississauga Ontario, L5N 3R7). The respective coefficients of variation were 3.62% and 1.30%. The FTI was reported as the value of the RT3U times T4 concentration/100 which reflects the quantity of free T4. Measurement of total triiodothyronine, (T3) was done manually using a radioimmunoassay (Tritab RIA, Organon Tenicka Corp., 2200 Eglington Ave. E., Scarborough, Ontario, M1L 2N3). The CV was 2.9%.

2.4 EXPERIMENTAL LABORATORY METHODS

2.4.1 Development of HPLC method for measurement of serum amiodarone and desethylamiodarone concentrations

No local clinical laboratories were available to measure amiodarone concentrations at the time of the study. A new assay was developed to improve sample preparation techniques for rapid accurate processing of samples of small volume. Amiodarone and its major metabolite, N-desethylamiodarone (DEA), have been assayed by several liquid chromatographic methods. The assays of Andreasen et al (1981), Brien et al (1983), Plomp et al (1983) and Weir et al (1985) which used a crude protein precipitation were investigated first. These assays did not sufficiently purify the samples, causing extra peaks in the chromatograms. The assays of Lesko et al (1981), Storey et al (1982), Debbas et al (1983), Duranti et al (1983) and Mostow et al (1983) which used solvent extraction techniques produced cleaner results, but were found to be cumbersome and had inconsistent analytical recoveries. The goal in developing a new assay was to increase sensitivity and decrease the sample volume required by improving the sample preparation. This was accomplished by applying the technique of solid-phase extraction and chromatographing the extracts on short efficient columns. This new method has been published (Pollak et al 1986).

Stored frozen serum was thawed and vortexed to distribute the protein evenly. Solid-phase cyano bonded (CN) minicolumns were prepared in a vacuum extraction manifold by washing with 1 mL of methanol followed by 3 washes with 1 mL of double distilled water. Internal Standard (Labaz L8040 - see Figure 1-5) prepared as 50 µg/mL was added in the proportion of 100 µL to

1 mL of water on the prepared column. A 100 µL aliquot of serum was added to this water and allowed to pass through the minicolumn under low vacuum. The column was washed 3 times with 1 mL water and once with 1 mL of 50:50 methanol:water. Drugs retained on the minicolumn were eluted with 0.1% triethylamine (TEA) in methanol. The eluent was dried under a flow of nitrogen and reconstituted to a volume of 100 µL with 50:50 acetonitrile:water. The sample was then loaded with standards and controls into an automated sampling tray (Waters Intelligent Sample Processor, Waters Millipore). Chromatography of 20 µL aliquots of extracts was done on a 5 x 0.4 cm column packed with C18 NovaPak 5 µm beads. A mobile phase of 60:40 acetonitrile:diphosphate buffer (pH 3.0) with 0.05% TEA was run at 1 mL/min. The effluent was monitored at 242 nm (maximum optical absorbance of amiodarone).

The ratios of amiodarone and DEA to internal standard were related in a linear manner between the concentrations of 250 and 8000 µg/L. The within run coefficient of variation was 3.7% for amiodarone and 5.0% for DEA. The between run coefficient of variation was 8.3% for amiodarone and 5.7% for DEA. Absolute recovery was determined by comparing the average peak area for six extracted sera at each standard concentration of amiodarone/DEA (from 250 to 8000 µg/L) with that of unextracted samples of identical concentrations made up in reconstituting fluid. The mean and standard errors for the absolute recoveries were 85 ± 3.8% for amiodarone and 87 ± 4.5% for DEA. Interference by common cardiovascular medications with a potential for co-extraction with amiodarone (weak bases) was checked by attempting to chromatograph solutions of these medications under the same conditions as amiodarone. Of the 17 drugs tested, only bepridil co-eluted with a peak of interest, that of DEA. This drug was not used by any of the patients followed in the pilot study.

2.4.2 Measurement of superoxide dismutase activity

Superoxide Dismutase (SOD - E.C. 1.15.1.1, MW 32,500, 2 Cu^{2+}, 2 Zn^{2+}) enzyme activity was determined by a modification of the pyrogallol autoxidation method developed by Marklund and Marklund (1974) which was published by Del Maestro and McDonald (1984). The frozen lysed erythrocytes were thawed and 10 µL added to 3 mL of 50 mM Tris-HCl buffer, pH 8.2 containing 1 mM diethlyenetriamine-pentacetic acid (DTPA) maintained at 25° C in a water bath and aerated by vigorous stirring. Pyrogallol (1,2,3, benzetriol, MW=126.11 Daltons) was added to 0.01 M HCl to produce a solution of 3.0 g/L equal to 24 mM concentration. The reaction was initiated by adding 25 µL of this pyrogallol solution to the erythrocyte solution to produce a 0.2 mM concentration of pyrogallol in the reaction vial. Oxidation of pyrogallol was detected by monitoring optical density at the 420 nm wavelength. Bovine CuZn SOD (Sigma) was used to construct a standard reaction curve which was acceptably linear up to 75% inhibition (as in Del Maestro and McDonald 1984). The percent inhibition was calculated from the slope of reaction rate as monitored by spectrograph. The ordinate of the experimental sample reaction slope (E) at three minutes was compared to the ordinate of the standard reaction slope (S). The equation $\left(\frac{1 - E}{S}\right)100\%$ yields the percent inhibition of pyrogallol autoxidation by the SOD in the sample. Samples producing slopes sufficiently flat to yield an experimental ordinate less than 25% of the standard slope ordinate (i.e. $\frac{E}{S} < 0.25$) were considered unacceptable and were rerun with the sample diluted by half to increase the rate of reaction to an acceptable range. The reaction system was tested with amiodarone and DEA to verify that the reaction rate was not influenced by either compound contained in the erythrocytes.

The activity of SOD was converted to ng of standard SOD knowing that 50% percent inhibition of pyrogallol autoxidation occurs with 128 ng/L of SOD. The equation: $SOD = (\%\text{inhibition}) \cdot \frac{128}{50} \cdot \frac{3 \cdot 1000}{(\text{final sample dilution})}$ provides the activity in terms of ng of standard SOD. This value was then divided by the concentration of hemoglobin in the sample calculated from the optical absorbance at the three wavelengths; a (380 nm), b (415 nm) and c (450 nm) using the equation:

$Hb = \frac{(2a - b - c)}{1.655 \cdot 0.01319}$ mg/dL.

The results were recorded as SOD ng/mg-Hb.

2.5 DATA ANALYSIS

Clinical data were recorded in the patient's chart at each visit and the observations of interest were transferred to a clinical flow sheet. These data along with results obtained from the clinical and experimental laboratories were entered into a data base on a MacIntosh Computer (Apple Canada Inc., Toronto Ontario). The data were then sorted to create files for each parameter of interest and inspected regarding the degree of change in each parameter. The following standard statistical procedures were then applied to the analyses of the data (Snedecor and Cochran 1980). For each parameter, data from all of the follow-up visits were simultaneously compared with the baseline values using a two way analysis of variance (repeated measures design). The interaction term, time*subject, was not expected to be statistically significant and was pooled with the experimental error term (residual sum of squares). Any further comparisons between follow-up and baseline data were made using Fisher test of least significant difference (LSD). Lines were fitted to mean data using a least squares linear regression for the number of points identified in each case, in order to obtain an estimate of the degree of correlation between the two

parameters over that range of values. Analyses of subpopulations were undertaken for exploratory purposes to identify whether possible relationships between the development of toxicity and other measured parameters had been masked in the analyses of the total population. Such analyses were not used to prove hypotheses, but to uncover relationships which might be further tested in future experiments. John Tukey (1977) stresses in his book on exploratory data analysis that failing to make such analyses frequently overlooks the most interesting results of an experiment and that the design of future experiments using confirmatory statistics is greatly aided by the results of exploratory data analyses. As stated by Furberg and Byington (1983): "The investigators of a clinical trial usually conduct subgroup analyses to determine whether certain groups of patients differ from others." In the current clinical trial, the criteria for the selection of subgroups used in further analysis was based on the development of clinical or subclinical toxicity as defined prospectively in the methods section. An unpaired t-test or χ^2 test were used for comparisons of parameters such as demographic data between the selected subpopulations at specified time points. Results are expressed in the text as means ± standard error (SE) with the statistical probabilities reported as the probability (p) for the appropriate test. Probabilities less than 0.05 were considered statistically significant. Statistical calculations were performed using the Statview 512+ software package. Graphs presented in the figures represent mean values with SE bars. Errors bars are omitted where they confuse the interpretation of the graph such as with log scales for which they are asymmetrical around the means and vary in magnitude over the range of values.

CHAPTER 3 - RESULTS

3.1 PATIENT ENROLLMENT

Thirty-eight patients were enrolled in the study and followed between February 14, 1984 and December 10, 1986. Patient identity code numbers were not reassigned if the patient was disqualified on the basis of abnormal initial laboratory results or dropped out. Seven subjects did not complete follow-up for the following reasons. Subjects 1, 2 and 3 were lost to follow-up without opportunity for reassessment because of transportation problems. Subject 16 was excluded because of thyroid abnormalities on baseline testing. Subjects 26 and 30 died after 2 and 3 months of follow-up respectively. Enquiries to their family physicians indicated that autopsies were not obtained and only cardiac arrest was recorded as the cause of death. Both patients had pre-existing cardiac ischemic events, making the probable cause of death either cardiac infarction or arrhythmia. Subject 32 died of arrhythmia in hospital before initial assessment was completed. The characteristics of the study patients are summarized in Table 3-1 and the subjects listed in Table 3-2.

Table 3-1. Characteristics of patients followed in the study

Disposition	Number	Percent
Number Entered	38	—
Disqualified	1	2.6
Lost to follow-up	3	7.9
Died	3	7.9
Followed 1 yr.	31	81.6
Characteristics of patients followed for one year.	**Number**	**Percent**
Male	23	74.2
Female	8	25.8
Average Age (yr)	55.2 ± 2.4	—
Average Wt. (kg)	81.2 ± 3.1	—
Average Daily Dose (mg)	379 ± 17	—

Table 3-2. List of 31 patients completing follow-up of one year

I.D. Code	Initials	Age (y)	Sex	Wt. (Kg)	Dose (mg/d)	Arrhythmia	Primary Diagnosis
04	WM	54	M	81	400	A Fib	Tachy/Brady Syndrome
05	BF	68	M	80	200	A Fib	Ischemia
06	SB	48	F	120	400	PAT	-
07	SG	36	F	90	300	VT	-
08	MG	49	F	82	400	VT	RV dysplasia
09	WS	56	M	68	300	PAT	Wolff-Parkinson-White
10	RB	29	M	89	400	A Fib	Hypertrophic CM
11	DH	46	M	68	200	PAT	Wolff-Parkinson-White
12	JT	68	M	76	200	A Fib	-
13	JG	63	M	80	400	VT	Aortic valve insufficiency
14	AW	65	F	76	300	PAT	Tachy/Brady Syndrome
15	HC	72	F	51	400	PAT	Atrial septal defect
17	AB	66	M	91	400	A Flut	Rheumatic Heart Disease
18	CL	52	M	75	300	VT	Ischemia
19	LC	72	M	64	400	VT	Ischemia
20	BM	50	M	98	400	A Flut	-
21	JO	67	M	79	400	VT	Ischemia
22	LM	58	F	89	200	VT	Ischemia
23	IK	25	M	66	400	VT	RV dysplasia
24	NT	68	M	70	400	VT	Ischemia
25	JM	40	M	125	400	VT	Congestive CM
27	DM	53	M	68	600	VT	Ischemia
28	DG	37	M	82	400	PAT	-
29	JL	65	M	76	400	VT	Ischemia
31	IM	53	F	115	400	PAT	Hypertrophic CM
33	ML	63	F	71	400	A Fib	-
34	GB	75	M	63	400	VT	Ischemia
35	JH	58	M	70	400	VT	Ischemia
36	DD	49	M	82	400	A Fib	Hypertrophic CM
37	JO	68	M	78	600	VF	Ischemia
38	CH	51	M	92	400	VT	Ischemia

PAT = paroxysmal atrial tachycardia A Fib = atrial fibrillation A Flut = atrial flutter

VT = ventricular tachycardia VF = ventricular fibrillation CM = Cardiomyopathy

Oral therapy with amiodarone was adjusted by clinical evaluation of arrhythmia suppression. The majority of patients were continued on a maintenance dose of 400 mg/day after an initial period of augmented doses consisting of 1600 mg/day for one week. Eighteen remained on 400 mg/day for the year, 4 were maintained on 300 mg/day, 4 on 200 mg/day, and 2 were increased to 600 mg/day. Three other patients taking 400 mg/day stopped the medication for 1 to 3 months. Subject 10 was of special interest because he had been followed previously for 6 months while taking amiodarone but had stopped the medication for 6 months in order to undergo surgery. Data were then collected on him for the year after he restarted therapy with amiodarone.

The demographic data used in the comparisons of subgroups found in the results are listed in Table 3-2 and again in Table 3-4.

3.1.1 Drug concentrations

The mean and individual serum concentrations of amiodarone and DEA for the 31 patients followed are presented in Appendix 5. The mean concentration of amiodarone rose to 2.5 ± 0.25 mg/L at 6 months of therapy with no statistically significant changes in the last 6 months. A wide intersubject variation in amiodarone concentration is apparent from inspecting the individual concentration-time curves in Appendix 6. The mean serum concentration of DEA steadily increased throughout the year to a maximum value of 1.4 ± 0.11 mg/L at the 12 month assessment. An intersubject variability smaller than that for amiodarone concentrations is manifested in the smaller standard errors for the mean DEA concentration. Both the mean and individual DEA concentration-time profiles exhibited less tendency to undulating concentrations

than the profiles obtained for amiodarone. The ratio of mean DEA to mean amiodarone serum concentrations remained constant at approximately 0.5 from the first month onwards.

For the purposes of analysing the relationship between dose administered and serum concentration and the estimation of elimination rate from concentrations in the accumulation phase a data set which excluded the concentrations from four subjects was created. Excluded were the three subjects who were not accumulating drug during the whole study and the one with a residual serum amiodarone at study entry. Fig. 3-1 illustrates the amiodarone and DEA mean concentration-time profiles for this data set.

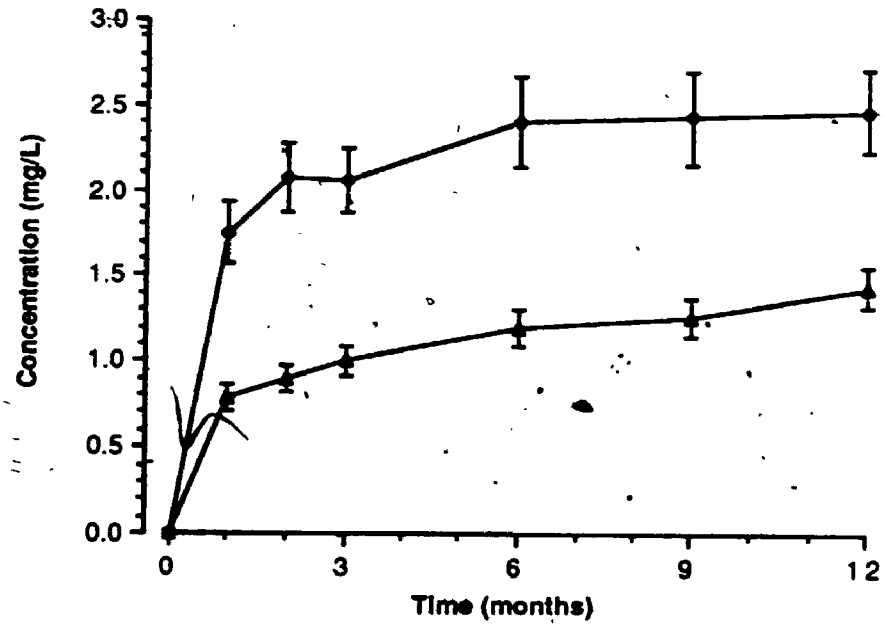

Figure 3-1. Mean serum drug concentrations ± SE in 27 subjects receiving continuous amiodarone therapy for one year.

Symbol key: ◆ amiodarone, ▲ desethylamiodarone (DEA).

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Dose-concentration relationships were observed for both serum amiodarone and DEA concentrations during the first year of therapy with amiodarone. In Fig. 3-2 a semi-log plot of the mean serum concentrations of amiodarone and DEA vs. log mean accumulated dose per kilogram of body weight revealed a good linear relationship for serum amiodarone. Least squares regression through six mean concentration points produced the equation $y = -0.110 + 0.810x$, $r^2 = 0.93$, $p<0.002$. Least squares regression through six mean serum DEA concentrations produced a relationship with a higher degree of correlation described by the equation: $y = -0.930 + 0.710x$, $r^2 = 0.98$ ($p<0.0001$).

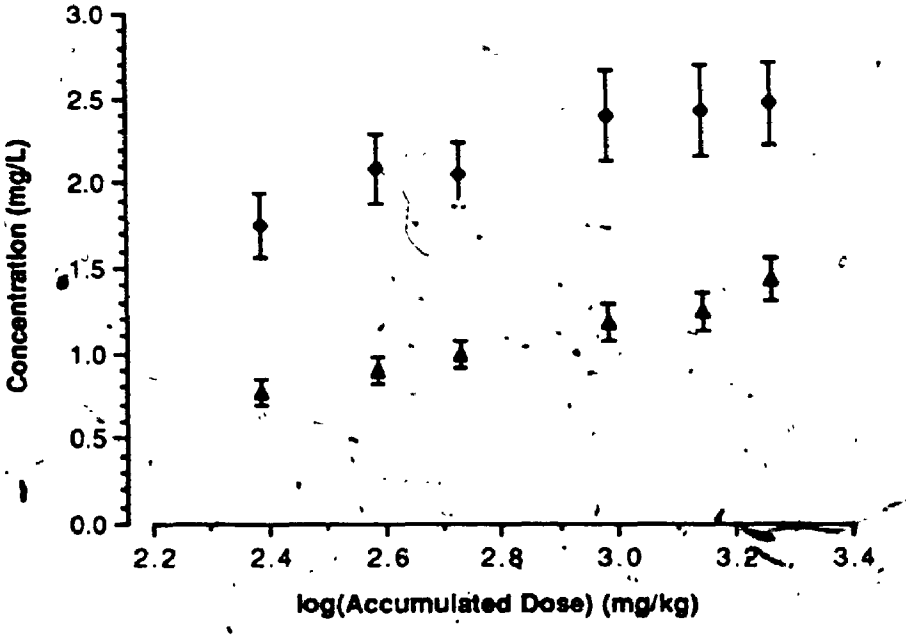

Figure 3-2. Mean serum drug concentrations vs log accumulated dose per kg body weight in 27 patients on amiodarone.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ◆ Amiodarone, ▲ DEA. Least squares linear regression through six mean concentration points produced the equation $y = -0.110 + 0.810x$, ($r^2 = 0.93$; $p<0.002$) for amiodarone and the equation: $y = -0.930 + 0.710x$, ($r^2 = 0.98$, $p<0.0001$) for DEA.

As explained in detail in Appendix 7, when a drug is administered at a consistent dose and dosing interval, its serum concentrations are mathematically related to the time since administration began until steady state is achieved at the end of the accumulation phase. This relationship between concentration and time during the accumulation phase can be used to estimate the elimination half-life of the drug. When all dosing factors are held constant, a plot of the natural log of $\left[\frac{(C_{ss} - C)}{C_{ss}}\right]$ vs. time, where C is the plasma concentration at any time (t) and C_{ss} is the steady-state concentration, will yield a linear segment. The slope of this linear segment is equal to the terminal elimination constant (k).

The final mean amiodarone and DEA concentrations were used as approximations of those in steady-state, realizing that it could not be determined with certainty that either concentration had completely stopped rising at 12 months. Because C_{ss} is a constant, its magnitude only shifts the intercept of the linear segment and the accuracy of the estimate of steady-state concentration does not affect the slope of the concentration-time relationship. Using these estimates of steady-state concentrations, the natural logarithms of the values $[(C_{ss} - C)/C_{ss}]$ were calculated for the mean concentrations of both amiodarone and DEA at each time point.

A plot of these calculated values against time is displayed in Fig. 3-3. As expected for first order drug elimination, a linear segment was observed. Least squares linear regression through the five points of this segment from the first to ninth months, produced equations for the amiodarone data with a slope of -0.37 ± 0.046 month^{-1} (r^2 =0.96) and for the DEA data with a slope of -0.16 ± 0.014 month^{-1} (r^2 =0.98). Given that these observed slopes represent k and that half-life equals $\frac{\ln(2)}{k}$, the estimated elimination half-life for amiodarone was 56.4 days (range of ±1SEM is 50.2 to 64.5 days) and for DEA 129 days (range of ± 1SEM is 119 to 141).

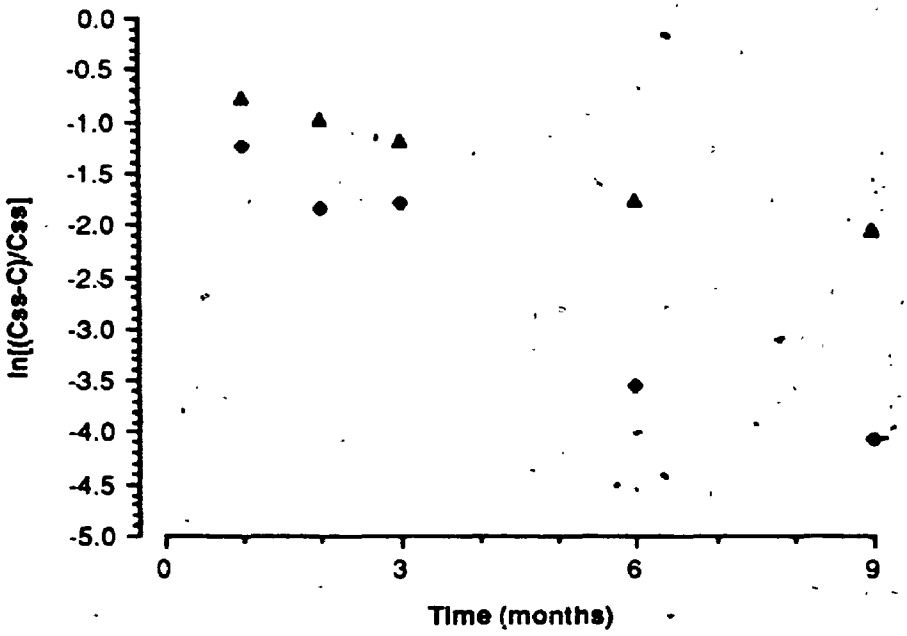

Figure 3-3. Estimation of elimination constants for amiodarone and DEA in 27 patients.

Observations from follow-up visits at 1,2,3,6 and 9 months.

Symbol key: ◆ Amiodarone, ▲ DEA . Least squares linear regression through five calculated points (natural logarithms of the values [(C_{ss} - C)/C_{ss}])produced a slope of -0.37 ± 0.046 month^{-1} (r^2 =0.96) for amiodarone and -0.16 ± 0.014 month^{-1} (r^2 =0.98) for the DEA.

From Fig. 3-1 it is apparent that the mean serum concentrations of DEA approximately paralleled those of amiodarone throughout the period of follow-up. DEA concentrations were mathematically related to those of amiodarone by a mean ratio of 0.5 over the year. Therefore the mean concentration-response profiles for amiodarone also paralleled those of DEA, being shifted in position to two times the concentration. One aim of the study was to demonstrate relationships between drug effects and serum drug concentrations. Since the drug concentration-response profiles for amiodarone and DEA paralleled one another, only one of the curves is illustrated in each of the results sections. The curve for DEA was chosen in each case because there was less intersubject variability and less undulation in the accumulating concentration curve for DEA making the concentration-effect relationships more readily apparent. Also the DEA metabolite is known to be pharmacologically active (Nattel 1986) providing prior reason to suspect that concentration-response curves exist for DEA.

3.1.2 Cardiac effects

The mean pulse rate at baseline for the 31 patients followed in the study was 86 ± 4.7 bpm. During the first month there was a statistically significant fall to 66 ± 1.6 bpm ($p<0.0001$, two way ANOVA) followed by a downward trend to 62 ± 2.2 bpm at 12 months follow-up as illustrated on the following page in Fig. 3-4.

The mean corrected QT interval (QTc) from baseline electrocardiograms (ECG) of the 31 patients was 441 ± 6.62 msec. Fig. 3-5 shows a statistically significant rise to 481 ± 8.08 msec at one year ($p<0.0001$, two way ANOVA).

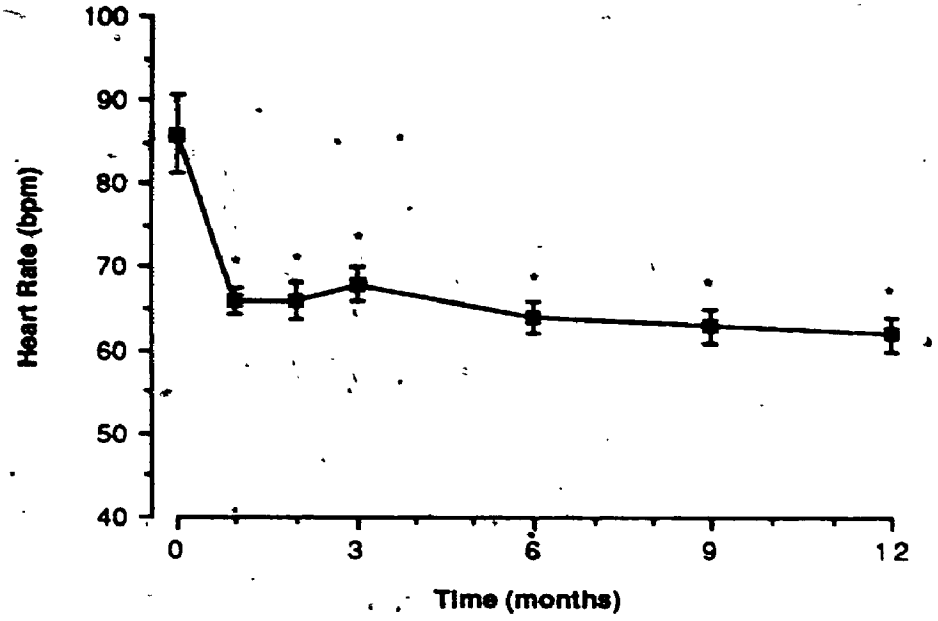

Figure 3-4. Mean heart rate ± SE in 31 patients receiving amiodarone over one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

* $p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

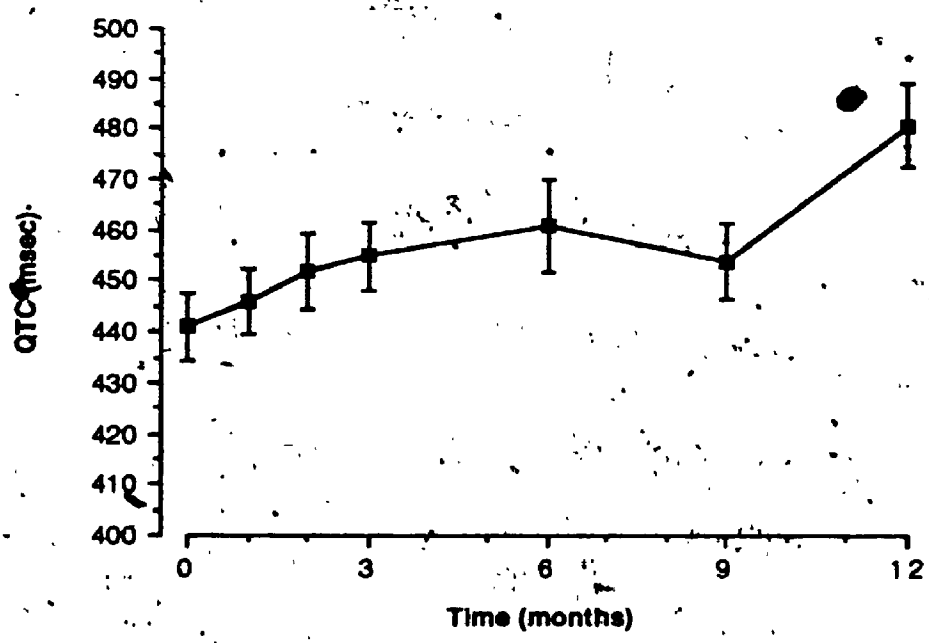

Figure 3-5. Mean QTc ± SE in 31 patients receiving amiodarone over one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

* $p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Least squares linear regression through first 4 points of the mean DEA concentration-response relationship for QTc in Fig. 3-6 reveals a correlation between mean QTc and log mean DEA concentration with $r^2 = 0.99$ ($p<0.006$).

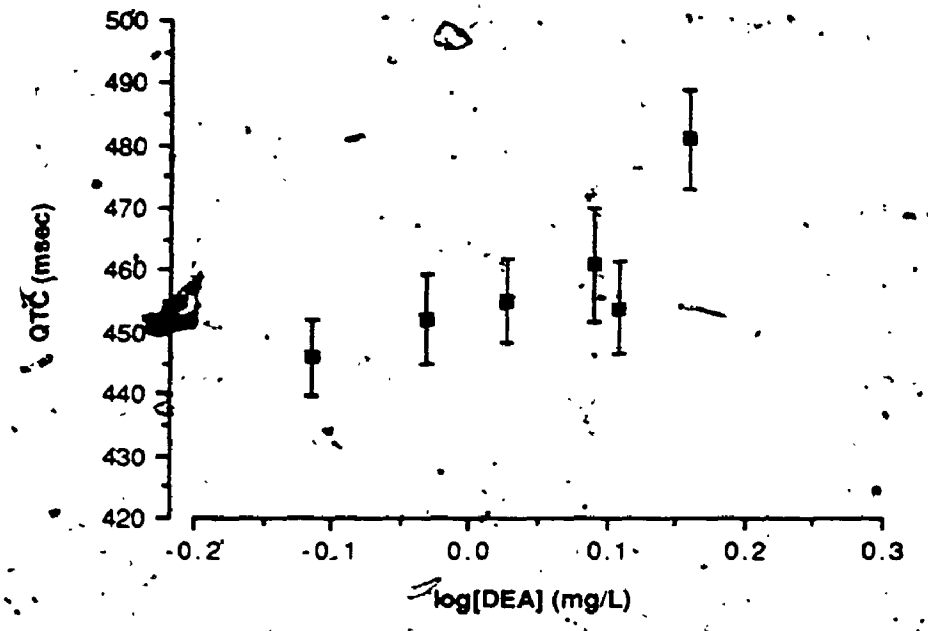

Figure 3-6. Mean QTc ± SE vs log mean serum [DEA] in 31 patients receiving amiodarone for one year.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.

Least squares linear regression through first 4 points $r^2 = 0.99$ ($p<0.006$).

Thirty-three patients presented for at least one reassessment including patients 26 and 30 who died outside of hospital and were considered as failures of arrhythmia control. This group was to estimate antiarrhythmic efficacy for this study. In the patients that completed the study, arrhythmias were suppressed early in follow-up except for the following cases. Patient 20 experienced recurrent atrial fibrillation on 200 mg and required an increase in dose to 400 mg daily. Patient 23 had recurrent ventricular arrhythmias in the first three months of follow-up controlled after increasing his dose form 400 mg to 600 mg daily for one month. Subject 27 required the addition of propafenone

after 5 months of follow-up in addition to 600 mg of amiodarone daily in order to control his arrhythmia. Patient 33 experienced atrial fibrillation while taking 200 mg of amiodarone requiring an increase to 400 mg daily for one month. Overall 27 of 33 patients (82%) leaving hospital on amiodarone were controlled during the first three months of therapy. Three of the above cases later achieved control with amiodarone alone so that eventually during the first year of therapy 30 of 33 subjects (91%) achieved control with amiodarone alone.

3.1.3 Ocular effects

A rapid increase in the mean grade of CMD in the first 3 months of therapy with a slowed but continued progression to 12 months is apparent in Fig. 3-7.

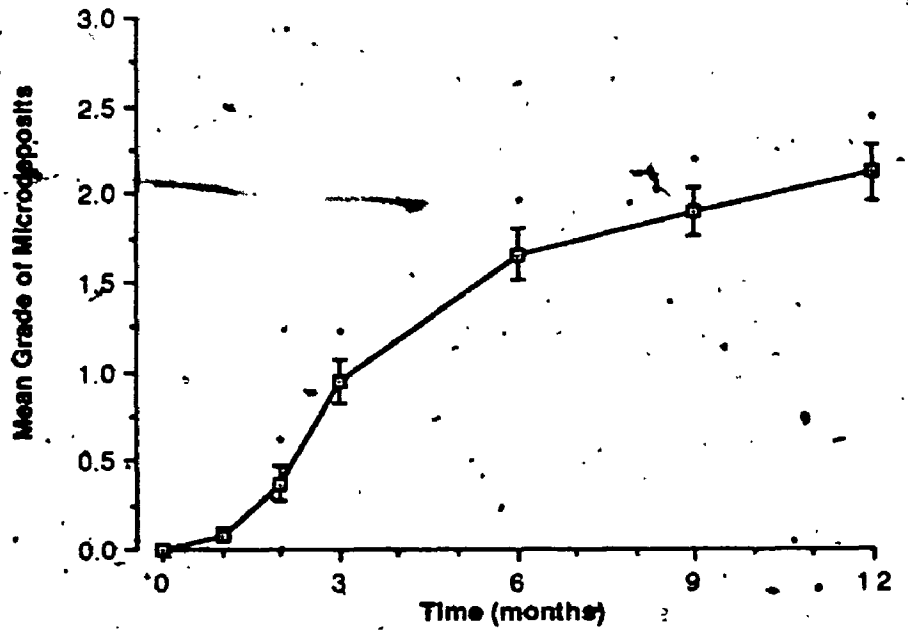

Figure 3-7. Mean grade of corneal microdeposits ± SE in 31 patients receiving amiodarone over one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

$p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Fig. 3-8 illustrates the rate of increase in the percent of patients with microdeposits. By 6 months of follow-up 100% of the subjects had some degree of corneal involvement. (One subject with trace deposits at six months returned to normal at 9 and 12 months.) Stratification into 4 grade intervals shows the accumulation of patients with higher grades of CMD as duration of therapy increased. Of these 31 patients with CMD, only one had symptomatic visual disturbances manifested as halos around bright lights when driving at night.

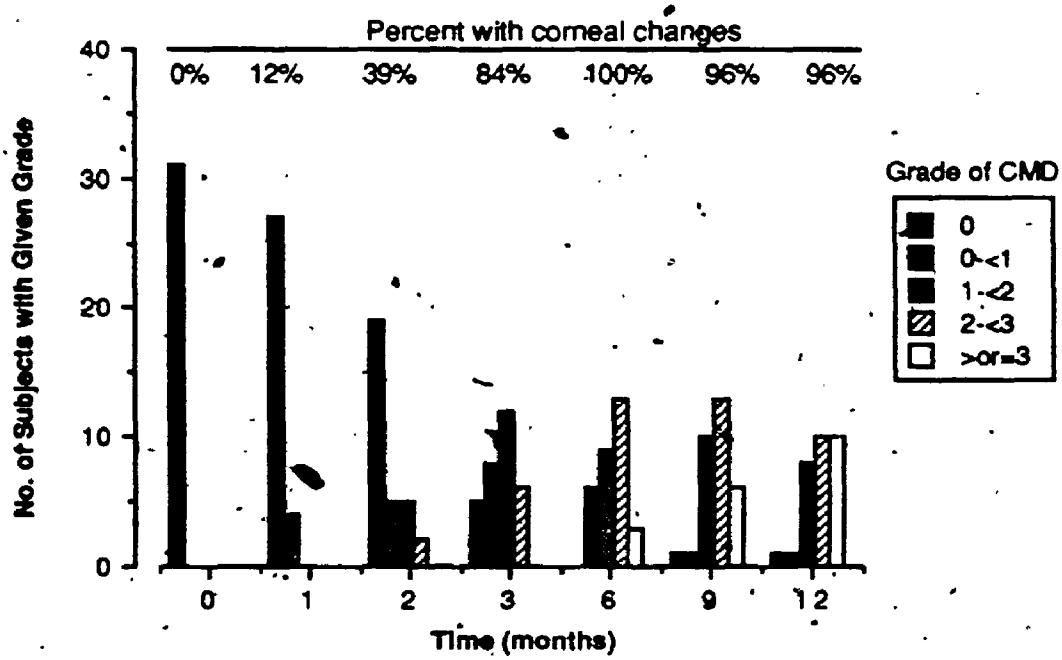

Figure 3-8. Corneal microdeposits stratified by grade in 31 patients receiving amiodarone over one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Top line lists the total number of patients with any grade of CMD expressed as a percent of the 31 patients followed with microscopic examination of the corneas during one year. Vertical bars represent the number of patients with no microdeposits and the number in each of 4 grade intervals for microdeposits as recorded at each follow-up.

A plot of the log mean DEA concentration-response relationship for corneal microdeposits illustrated in Fig. 3-9 reveals a sinusoidal curve. Least squares linear regression through the center four points of the curve demonstrates a correlation with $r^2 = 1.00$ ($p<0.001$).

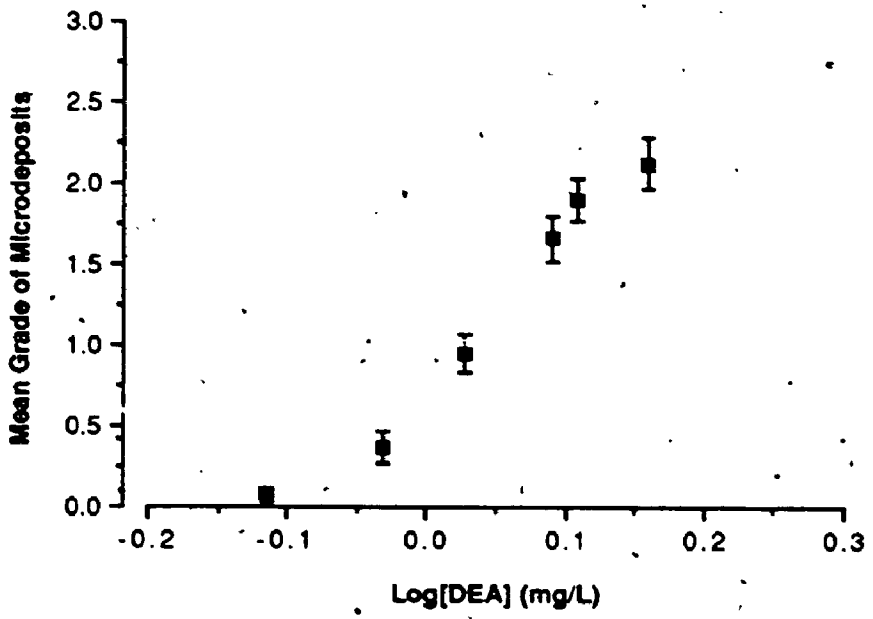

Figure 3-9. Mean grade of microdeposits ± SE vs log serum [DEA] in 31 patients receiving amiodarone for one year.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.
Least squares linear regression through the four points of the steep segment of the curve reveals a correlation with $r^2 = 1.00$ ($p<0.001$).

3.1.4 Pulmonary effects

Pulmonary function was followed in 30 patients. Data on patient 4 were lost because of organizational difficulties early in the course of the study. Chest radiographs were obtained for all patients every three months over the course of the one year follow-up. The radiologists assessing these films did not report the development of any interstitial lung disease suspected to be of drug induced

origin at any time during the study. There were no clinically important changes in lung volumes, expiratory flow rates, or arterialized capillary blood gases (PO_2 or PCO_2) in any of the patients.

There were no statistically significant changes in the mean DCO (diffusion capacity) for the 30 patients studied, but six patients had sustained falls in their DCO of greater than 20% and were therefore defined as having subclinical pulmonary toxicity. In order to explore differences between these 6 patients and those patients who did not develop toxicity, two groups were designated for further analysis. The 24 patients without toxicity were assigned to DCO Group 1 (D1) while the other 6 were assigned to group D2. Fig. 3-10 illustrates the mean values for DCO in the study population and in each of these groups.

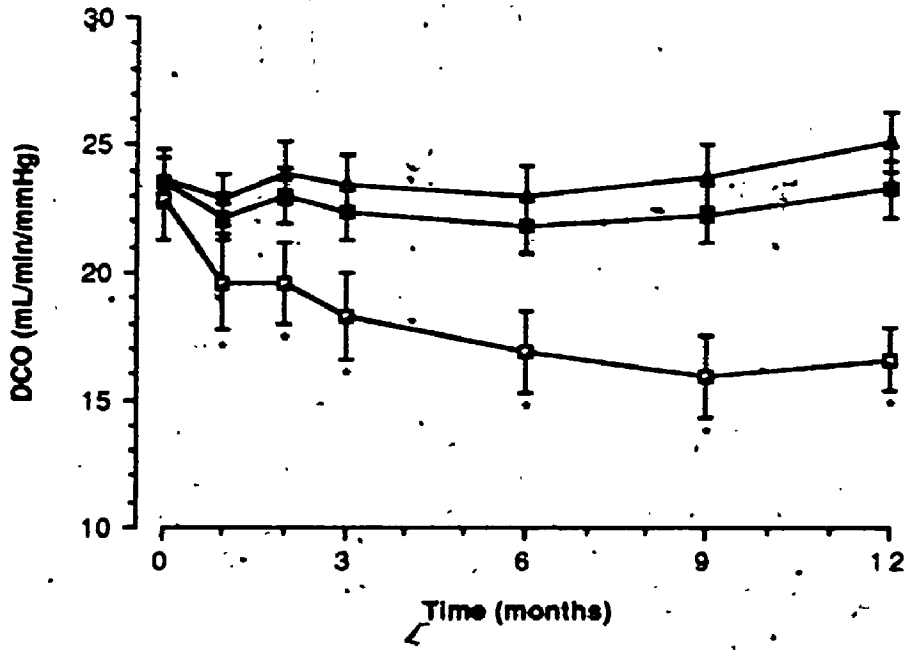

Figure 3-10. Mean DCO ± SE in 30 patients receiving amiodarone for one year and in subgroups with and without pulmonary toxicity.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Symbol key: ■ - Population followed (n=30); ▲ - Group D1 - no pulmonary toxicity (n=24); □ - Group D2 - subclinical pulmonary toxicity (n=6).

* $p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

The mean DCO values for patients in group D2, were statistically lower than baseline ($p<0.05$, two way ANOVA, Fisher LSD) from 3 to 12 months. The mean decrease in DCO from baseline for each visit was plotted against the log of mean DEA concentration, in order to analyze the concentration-response relationship. There was no relationship for group D1. The plot of the mean calculated decrease in DCO for Group D2 is illustrated in Fig. 3-11. Least squares linear regression through the four points of the rising segment of the curve shows a correlation with $r2 = 0.94$ ($p<0.01$).

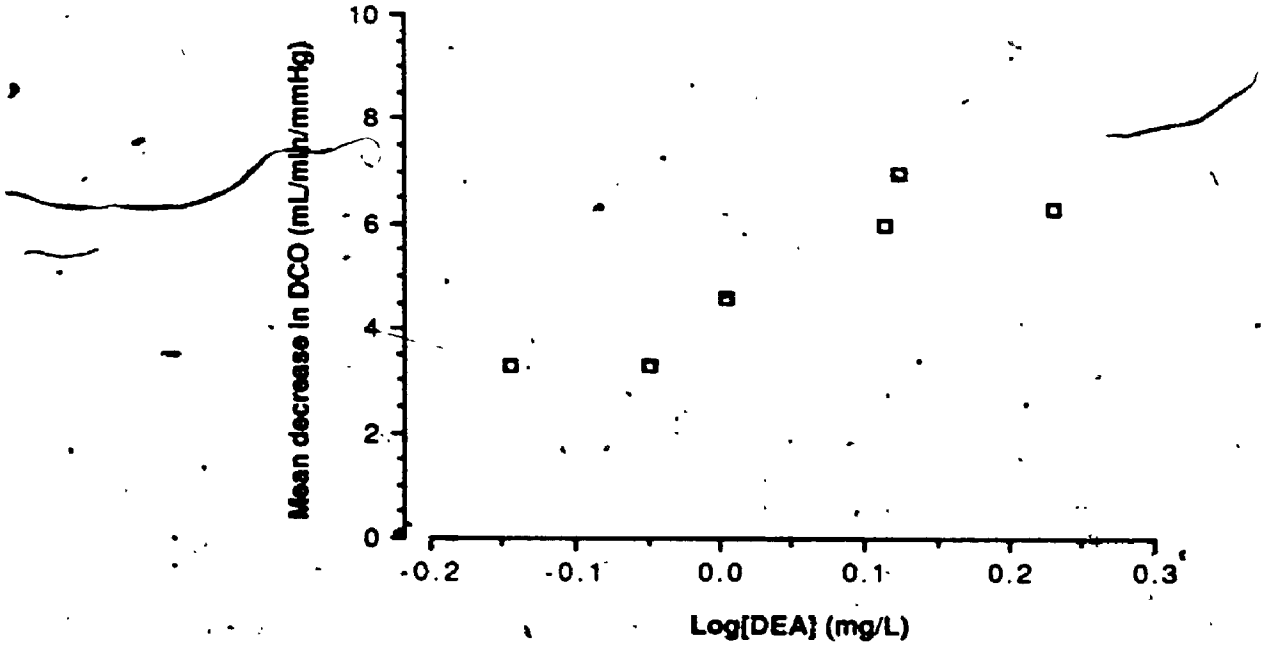

Figure 3-11. Mean decrease in DCO from baseline vs log [DEA] in 6 subjects with subclinical amiodarone pulmonary toxicity.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.
Mean decrease in DCO is the difference between mean baseline value and mean value at each visit. Error bars are omitted because of the derived nature of the values. Least squares linear regression through the four points of the rising segment of the curve shows a correlation with $r^2 = 0.94$ ($p<0.01$).

There were no statistically significant differences in age, weight, average dose/kg, serum DEA concentrations or baseline DCO between the two groups, but all subjects in the group developing subclinical pulmonary toxicity (D2) were males. All six patients in group D2 were clinically asymptomatic, had no changes in their chest radiographs and were continued on the medication without further decrease of their diffusion capacity after 9 months. One year after the completion of the study, information was obtained that patient 35, one of the six patients in group D2, developed dyspnea and radiographic changes diagnostic of overt amiodarone pulmonary toxicity. He was given alternative antiarrhythmic therapy and the pulmonary changes regressed.

3.1.5. Hepatic effects

One patient with abnormalities resulting from a retained common bile duct stone was excluded from analysis of liver function. In the 30 subjects analysed, the mean serum concentrations of ALT increased to 2.6 times baseline value ($p<0.04$, two way ANOVA), of AST to 1.8 times ($p<0.04$, two way ANOVA) and of ALK to 1.2 times ($p<0.05$, two way ANOVA) over the 12 months of follow-up. There were no cases of clinical jaundice and the mean bilirubin concentration remained constant. Eight of the 30 subjects analysed developed elevations in both serum ALT and AST to greater than twice baseline value fulfilling the criteria for hepatotoxicity set in the study design. To explore differences between patients developing toxicity and those not, two subgroups were defined. The 22 patients without toxic changes in hepatic enzyme concentrations were designated as Hepatic Group 1 (H1), while the 8 patients with hepatic toxicity were assigned to group H2. All the patients in group H2 were male. There were no differences in age, weight or mean dose/kg between groups.

Fig. 3-12 illustrates the statistically significant elevations of mean serum ALT concentrations in the overall population (n=30) and in both groups H1 and H2 ($p<0.05$, two way ANOVA, Fisher LSD). The subgroup in which concentrations of both transaminases doubled (H2) had a mean ALT which was above the upper limit of normal at baseline, was statistically higher than that in H1 at all time points ($p<0.02$, unpaired t-test) and had the most prominent increase.

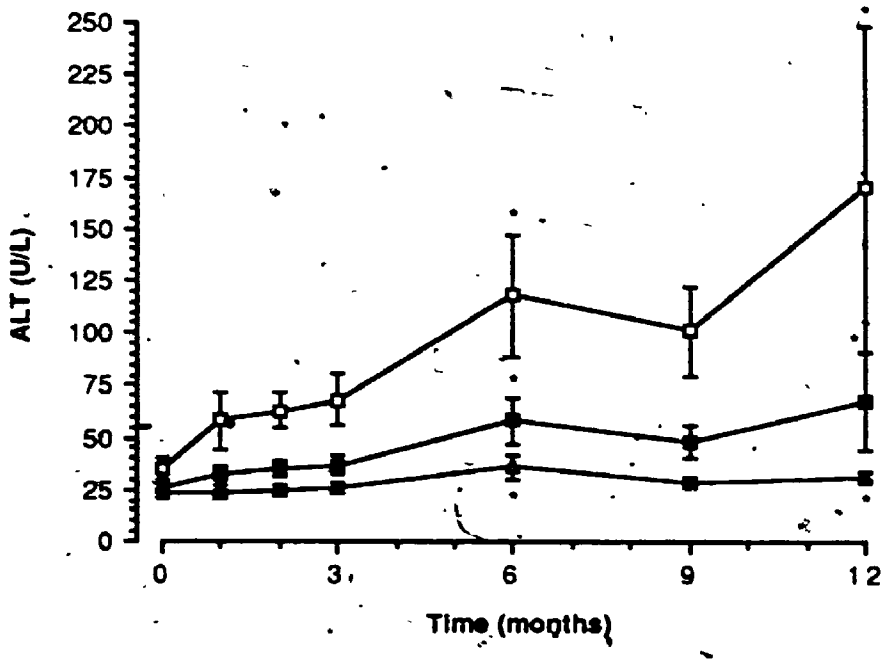

Figure 3-12. Mean serum ALT concentrations ± SE over one year in 30 patients and in subgroups with and without subclinical amiodarone hepatotoxicity.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ■ - all 30 subjects analysed.

▲ - Group H1 - subjects with no hepatotoxicity (n=22).

□ - Group H2 - subjects developing subclinical hepatotoxicity (n=8).

* $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Changes in AST paralleled those of ALT in group H2 but AST did not increase significantly in group H1. No sustained elevations of ALT were

observed in group H1. There was a statistically significant rise in the mean values for ALK in group H2 from 76.8 ± 6.1 U/L to 113 ± 27.5 U/L ($p<0.05$, two way ANOVA) but no change was observed in group H1.

Groups H1 and H2 also differed in mean serum DEA concentrations as is illustrated in Fig. 3-13. Man serum DEA concentration in group H2 with subclinical hepatotoxicity was statistically higher than in group H1 from 6 months onwards ($p<0.03$, unpaired t-test).

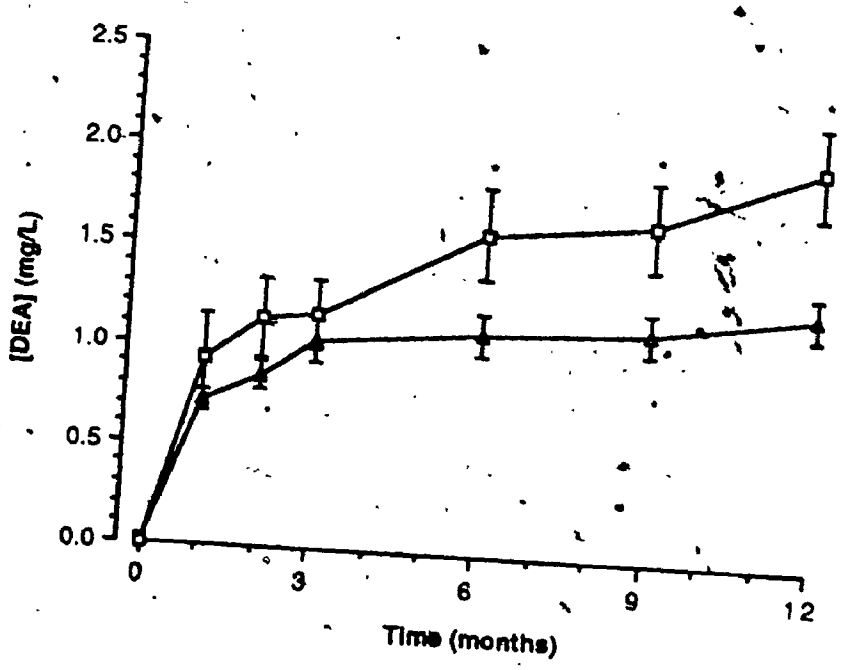

Figure 3-13. Mean serum DEA concentrations ± SE in subgroups with and without subclinical amiodarone hepatotoxicity.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Comparison of DEA concentrations between groups. Symbol key:

▲ - Group H1 - subjects with no hepatotoxicity (n=22).

□ - Group H2 - subjects developing subclinical hepatotoxicity (n=8).

* $p< 0.05$ difference between concentrations in the two groups at the indicated time point (unpaired t-test).

Fig. 3-14 illustrates relationships between the log mean serum DEA concentration and mean ALT concentration in the subgroups with and without hepatotoxicity. Least squares linear regression of the six points plotted for group H2 produced a correlation with $r^2 = 0.85$ ($p<0.009$) The slope of the least squares linear regression of the six points plotted for group H1 was not statistically different from zero. The mean ALT concentrations were lower in relation to any given mean DEA concentrations in group H1.

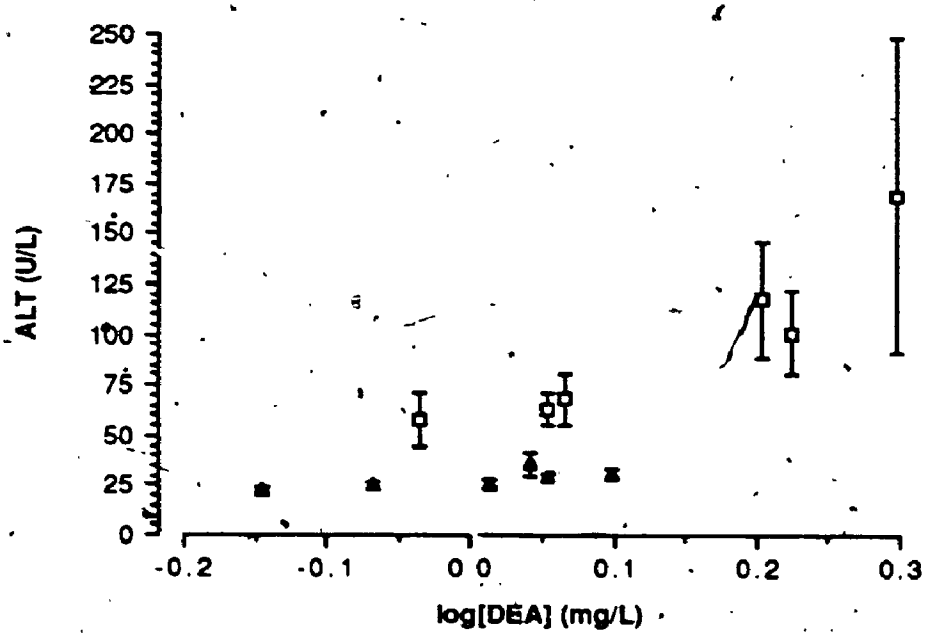

Figure 3-14. Mean serum ALT concentrations ± SE vs log mean serum [DEA] for subgroups with and without subclinical amiodarone hepatotoxicity.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.

Concentration-response relationship for mean serum ALT. Symbol key:

▲ - Group H1 - subjects with no hepatotoxicity (n=22).

□ - Group H2 - subjects developing subclinical hepatotoxicity (n=8).

Least squares linear regression of the six points plotted for group H2 produced a correlation with $r^2 = 0.85$ ($p<0.009$) . The mean ALT concentrations were lower in relation to mean DEA concentrations in group H1 and did not show a statistically significant trend to change with increasing DEA concentrations.

3.1.6 Changes in cholesterol, triglycerides and glucose

Samples for the measurement of cholesterol, triglyceride and glucose concentrations were not collected until subject 9 was enrolled. Data were available for the analysis of these parameters in 26 patients (21 men and 5 women). The age, sex, weight and mean dose/kg of these 26 patients were not statistically significant different from that of the study population. The mean total serum cholesterol concentrations in the 26 patients analysed showed a statistically significant increase from a baseline value of 5.0 ± 0.25 mmol/L (194 ± 9.7 mg/dL) to 5.7 ± 0.25 mmol/L (219 ± 9.7 mg/dL) after two months of therapy with amiodarone ($p<0.01$, two way ANOVA). This level of elevation was maintained during the year of follow-up as illustrated in Fig. 3-15.

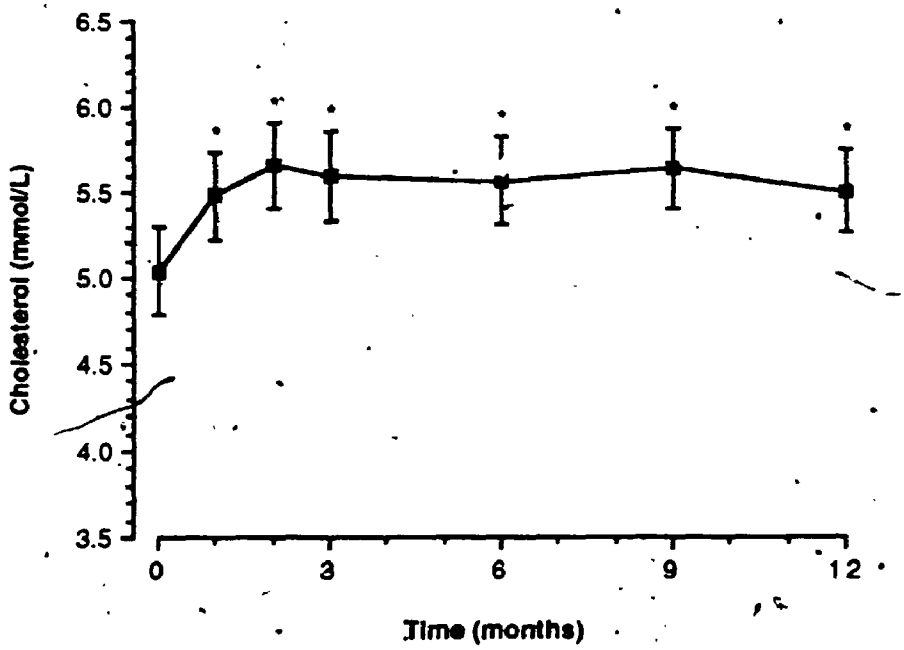

Figure 3-15. Mean total serum cholesterol concentrations ± SE in 26 patients receiving amiodarone for one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

* $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Normal serum cholesterol was considered to be a value below the 75 percentile for the age and sex of the patient as defined by the Lipid Research Clinics Reference Values (Rifkind and Segal 1983). Some patients with initially normal serum cholesterol concentrations developed values above the normal limit during therapy with amiodarone. In order to explore differences between these patients and those who did not develop abnormal cholesterol concentrations the patients were divided into groups first on the basis of their initial total serum cholesterol concentration. The 21 patients (19 M, 2 F) with normal initial cholesterol designated as Cholesterol Group 1 (C1), while Group C2 consisted of the 5 patients with abnormal initial cholesterol concentrations. The 21 patients in group C1 were then further distinguished on the basis of changes in their cholesterol. The 11 who remained normal throughout the follow-up were assigned to group C1a and the 10 patients who developed abnormal values for total serum cholesterol to group C1b. Two of the patients in C1b exceeded the 90 percentile during follow-up. There were no statistically significant differences in the mean age, sex or weight amongst the three groups (Table 3-3). Four patients in group C1a were receiving thiazides during the course of the study while only one patient in C1b received them. There were no perceived differences in diet between these groups.

Table 3-3. Characteristics of groups defined by cholesterol status

Group	C1a	C1b	C2
Initial cholesterol (percentile)	< 75	< 75	> 75
Maximum cholesterol (percentile)	< 75	> 75	> 75[†]
Number and Sex (M/F)	10/1	9/1	2/3
Age (years)	53.5 ± 4.0	58.7 ± 4.8	58.6 ± 2.5
Mean daily dose (mg/kg/day)	4.51 ± 0.34*	5.90 ± 0.60	4.33 ± 0.70*

* $p<0.02$ compared to group C1b [†]One patient on a 1200 kcal diabetic diet fell below the 75 percentile.

Group C2 which had abnormal initial cholesterol values had a mean baseline cholesterol concentration of 7.0 ± 0.26 mmol/L (271 ± 10.1 mg/dL) which remained unchanged throughout the year.

The mean total serum cholesterol concentrations for groups C1a and C1b are illustrated in Fig. 3-16.

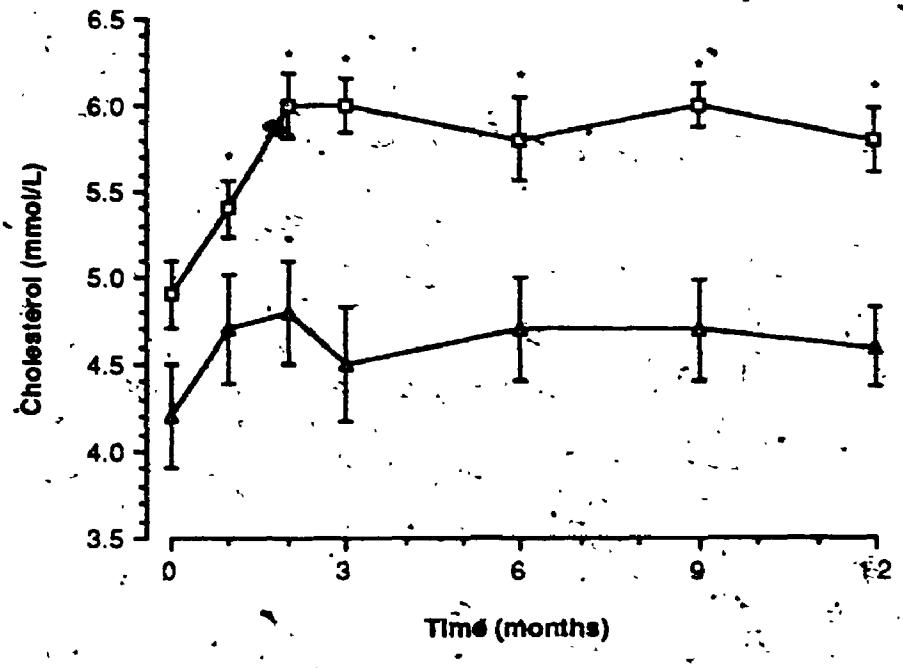

Figure 3-16. Mean total serum cholesterol concentrations ± SE for subgroups of patients with and without development of abnormal cholesterol concentrations on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Symbol key: ▲ - Group C1a - subjects with normal initial serum cholesterol who remained normal during the study (n=11). □ - Group C1b - subjects with normal initial serum cholesterol who developed abnormal values (n=10).

* $p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

In group C1a the initial total serum cholesterol of 4.2 ± 0.30 mmol/L (163 ± 11.6 mg/dL) rose 14% during the first 2 months to a value of 4.8 ± 0.30 (186 ± 11.6 mg/dL). The elevation at 2 months was statistically different from baseline ($p<0.05$, two way ANOVA), but subsequent values were slightly lower and not

statistically different from baseline. Group C1b, consisting of patients who developed abnormal cholesterol concentrations during the study, had a large increase of 22 % from baseline values. The mean cholesterol concentration in this group remained statistically different from baseline from the second through twelfth months ($p<0.05$, two way ANOVA, Fisher LSD). The initial total serum cholesterol in group C1b of 4.9 ± 0.20 mmol/L (190 ± 7.7 mg/dL) increased to 6.0 ± 0.19 mmol/L (232 ± 7.4 mg/dL) by the second month on amiodarone and remained at this level.

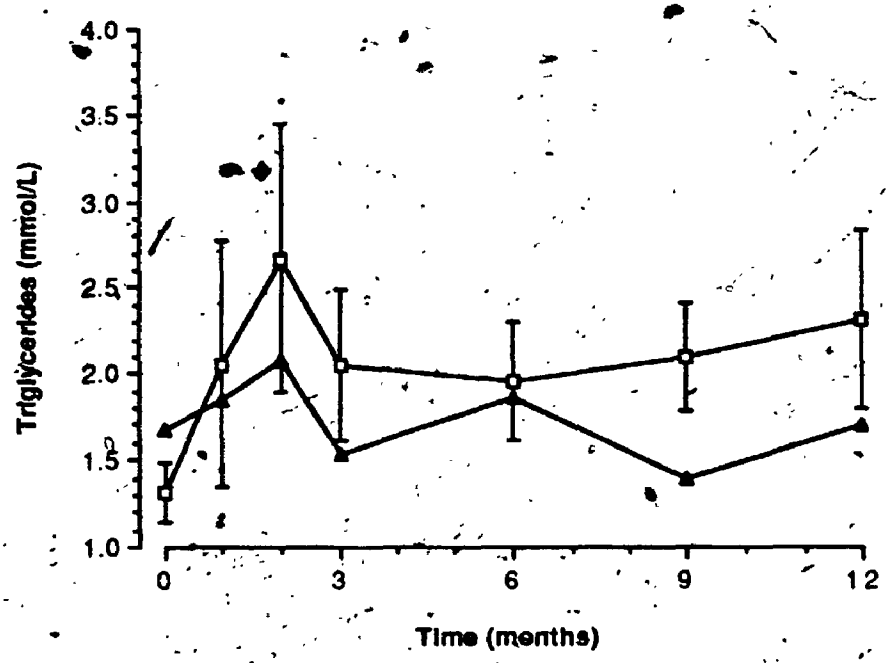

Figure 3-17. Mean serum triglyceride concentrations ± SE for subgroups of patients with and without development of abnormal cholesterol concentrations on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Because there is no change in C1a error bars are omitted for clarity in the graph. Symbol key: ▲ - Group C1a - subjects with normal initial serum cholesterol who remained normal during the study (n=11). □ - Group C1b - subjects with normal initial serum cholesterol who developed abnormal values (n=10). * $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).


2
1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
MicroD

The initial triglyceride concentration in the 26 patients was 1.58 ± 0.14 mmol/L (139 ± 12.4 mg/dL). A transient elevation occurring at one and two months was statistically higher than baseline ($p<0.05$, two way ANOVA, Fisher LSD), peaking at a value of 2.4 ± 0.33 mmol/L (213 ± 29.2 mg/dL). Group C1b (those developing abnormal cholesterol concentrations during the study) also had a transient but significant ($p<0.05$, two way ANOVA, Fisher LSD) elevation of triglycerides at two months (Fig. 3-17). No statistically significant changes occurred in triglycerides in either groups C1a or C2.

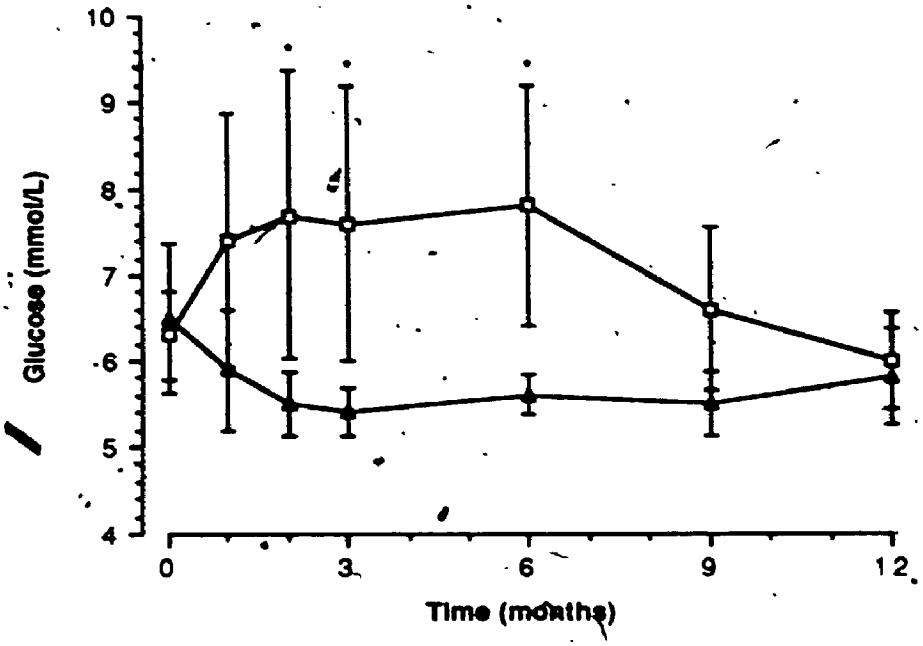

Figure 3-18. Mean serum glucose concentrations ± SE for subgroups of patients with and without development of abnormal cholesterol concentrations on amiodarone.
Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Symbol key: ▲ - Group C1a - subjects with normal initial serum cholesterol who remained normal during the study (n=11). □ - Group C1b - subjects with normal initial serum cholesterol who developed abnormal values (n=10).
* $p<.0.05$ of difference between groups (unpaired t-test).

There were no statistically significant changes in mean glucose concentrations during the study, but the concentrations of group C1b were statistically higher than group C1a ($p<0.05$, unpaired t-test) from months 2 through 6 (Fig. 3-18).

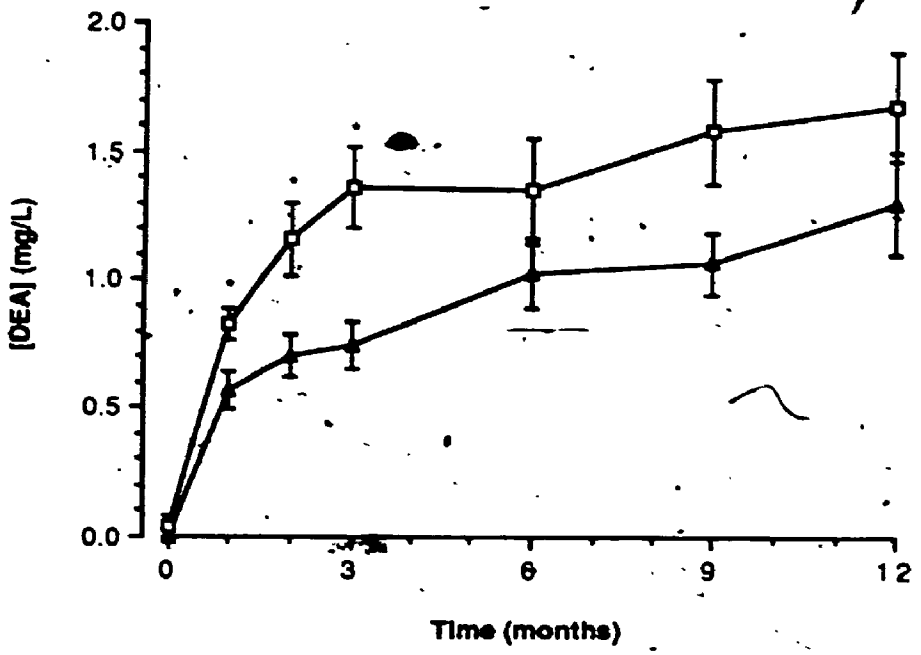

Figure 3-19. Mean serum DEA concentrations ± SE for subgroups of patients with and without development of abnormal cholesterol concentrations on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Symbol key: ▲ - Group C1a - subjects with normal initial serum cholesterol who remained normal during the study (n=11). □ - Group C1b - subjects with normal initial serum cholesterol who developed abnormal values (n=10).

* $p< 0.05$ of difference between groups (unpaired t-test).

A fourth dissimilarity was apparent between the two groups. Fig. 3-19 illustrates the difference in the mean serum DEA concentrations which occurred between groups C1a and C1b during the first 6 months of follow-up.. During this time, DEA concentrations in group C1b were statistically higher than in C1a ($p<0.05$, unpaired t-test).

The average daily amiodarone dose/kg body weight was also statistically higher ($p<0.02$, unpaired t-test) in group C1b than in C1a (6.0 ± 0.61 mg/kg and 4.3 ± 0.34 mg/kg respectively). However, in Fig. 3-20, differences in cholesterol concentration between the two groups were not demonstrated to represent the extremes of a continuous concentration-response relationship. Instead, the two groups displayed distinctly different patterns. In group C1b cholesterol concentrations were higher for a given DEA concentration and increased as DEA concentration increased during the first 2 months of the study. The cholesterol concentration did not vary with DEA concentration in group C1a after one month.

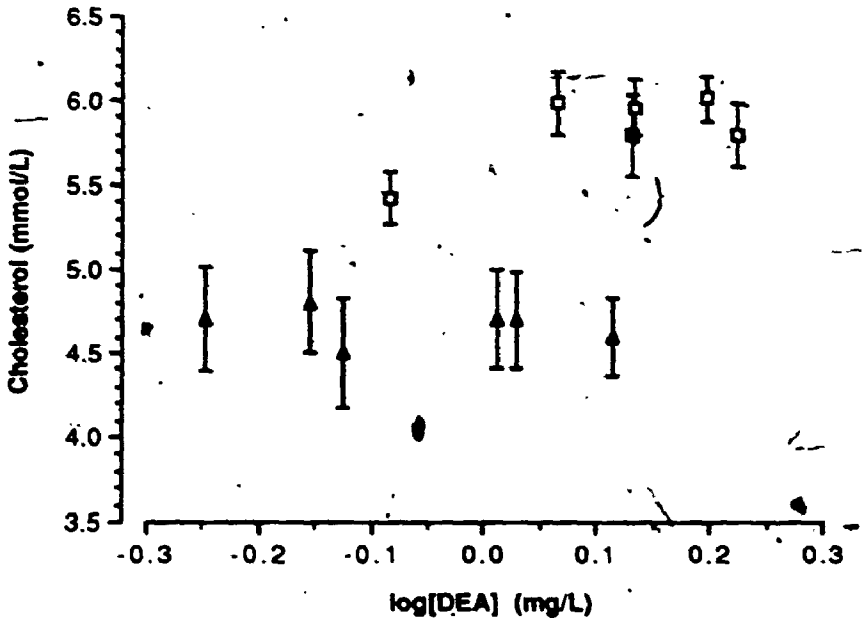

Figure 3-20. Mean serum cholesterol concentrations ± SE vs log mean [DEA] for subgroups of patients with and without development of abnormal cholesterol concentrations on amiodarone.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ▲ - Group C1a - subjects with normal initial serum cholesterol who remained normal during the study (n=11). □ - Group C1b - subjects with normal initial serum cholesterol who developed abnormal values (n=10). Patients in C1b had higher cholesterol concentration for a given [DEA].

3.1.7 Renal effects

There were no changes in serum sodium, potassium, chloride or bicarbonate concentrations over the year. Fig. 3-21 illustrates a statistically significant increase observed in the serum creatinine concentration from a baseline of 105 ± 3.6 mmol/L to 116 ± 3.9 mmol/L at 6 months of follow-up ($p<0.05$, two way ANOVA). Creatinine concentration remained at a level 10% above baseline for the rest of the 12 month assessment.

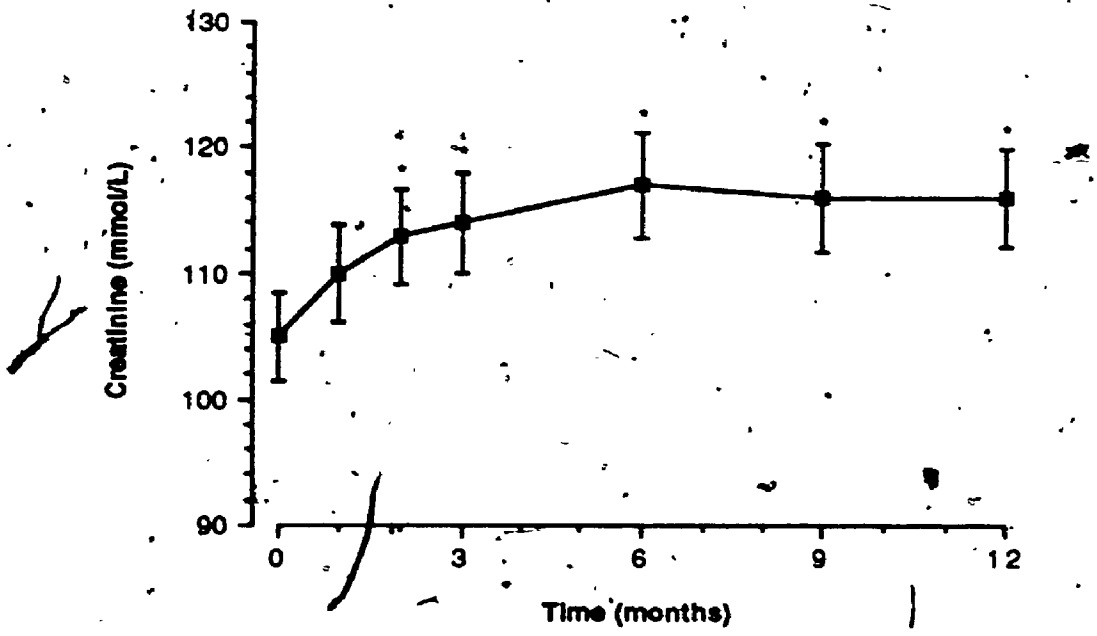

Figure 3-21. Mean serum creatinine in 31 patients receiving amiodarone over one year.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

* $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Least squares linear regression of the first four values of the log mean concentration-response relationship for creatinine (Fig. 3-22) shows a correlation over the first six months of the study with $r^2 = 0.98$ ($p<0.01$).

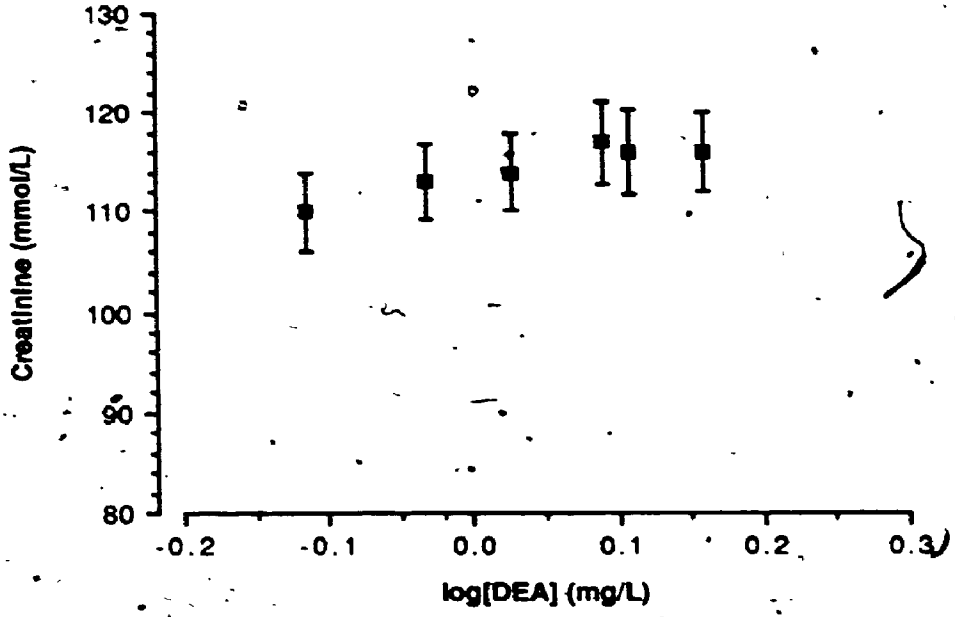

Figure 3-22. Mean serum creatinine ± SE vs log mean serum [DEA] in 31 patients receiving amiodarone over one year.
Observations from follow-up visits at 1,2,3,6,9 and 12 months.
Least squares linear regression through first 4 points $r^2 = 0.98$ ($p<0.01$).

3.1.8 Thyroid effects

One patient was hyperthyroid at baseline but was followed in the study under the supervision of an endocrinologist. Because he was not included in the analysis of thyroid effects, data from 30 subjects were analysed. Free Thyroxine Index (FTI) was used as the most sensitive indicator of changes in thyroid status. Resin uptake of T3 (RT3U) showed very little variation between

subjects or over time (Fig. 3-24). Therefore changes in the value of FTI were not attributable to fluctuations in the RT3U value used in deriving this index.

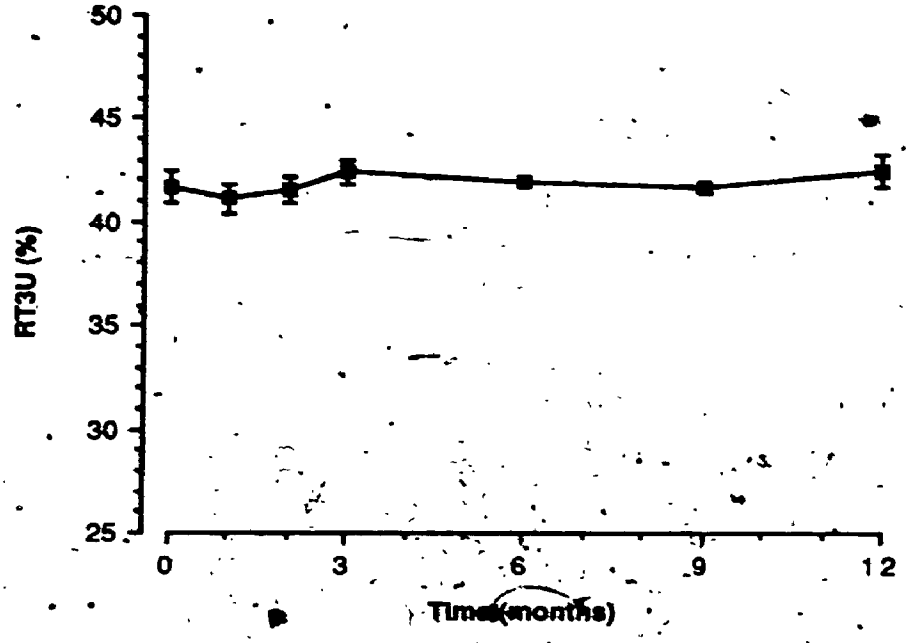

Figure 3-23. Mean RT3U ± SE in 30 patients on amiodarone 1 year.
Observations from follow-up visits at 1,2,3,6,9 and 12 months.

The mean baseline FTI for the 30 patients was 0.43 ± 0.012 units. Fig. 3-24 illustrates a statistically significant rise ($p<0.0001$; two way ANOVA) to 0.49 ± 0.016 after one month of amiodarone therapy with a further rise to 0.52 ± 0.019 at the third month. This elevation was sustained for the rest of the year of follow-up. The majority of patients (n=27) had FTI values which increased over the year. Of these, the 13 subjects who did not develop FTI values indicative of biochemical hyperthyroidism were designated FTI Group 1 (F1) while the 14 who developed abnormally high values of FTI (greater than 0,56) were designated as group F2. Group F3 consisted of 3 males who developed hypothyroid indices over the course of the study. This number was insufficient to produce reliable statistics. There were no statistically significant differences in the sex, age, body weight or average dose per kg amongst the thyroid groups.

Changes in FTI for each of these groups are also illustrated in Fig. 3-24. In group F1 the mean FTI did not change statistically from the baseline of 0.39 ± 0.019, but values showed a rising trend to 0.45 ± 0.016 at one month and slightly higher during the rest of follow-up. Group F2 had statistically significant elevations of FTI from a mean baseline FTI of 0.46 ± 0.011 rising to 0.58 ± 0.023 by the third month of follow-up ($p<0.0001$, two way ANOVA). This elevation was sustained at a level above the upper limit of normal from 3 months to the end of follow-up. Differences between groups F1 and F2 in the values of FTI were statistically significant at all observation times ($p<0.05$, unpaired t-test).

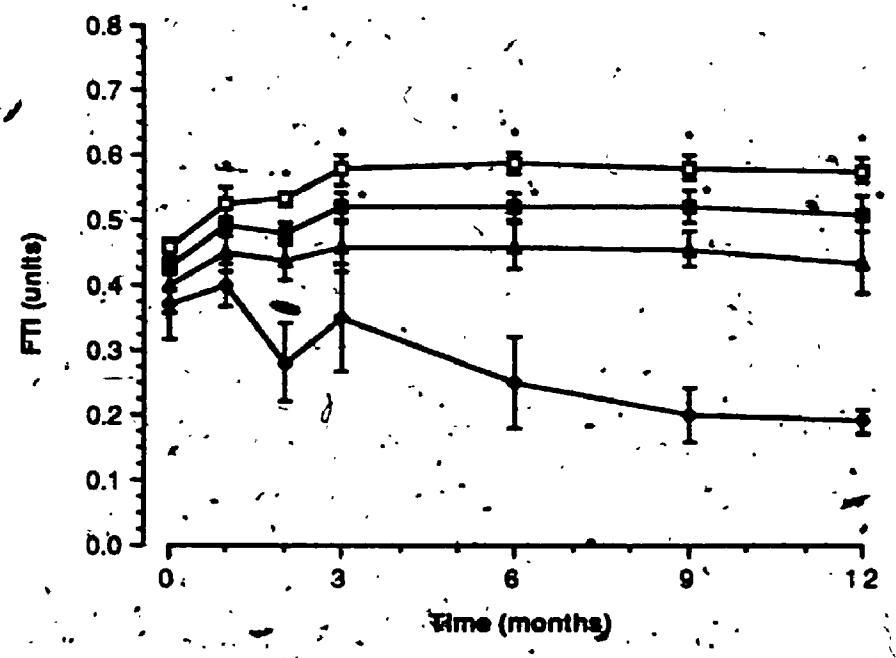

Figure 3-24. Mean free thyroxine index (FTI) ± SE for subgroups of patients with and without development of abnormal FTI values on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ■ - Mean values for all subjects available for analysis (n=30).

▲ - Group F1 - subjects with FTI remaining normal (n=13).

□ - Group F2 - subjects developing elevated FTI (n=14).

◆ - Group F3 - subjects who developed biochemical hypothyroidism (n=3).

* $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Mean thyroxine concentrations, as illustrated in Fig. 3-25, paralleled the values FTI in all groups but were less sensitive in indicating abnormal values without the scaling effect produced by RT3U in the FTI calculation. During follow-up, total T4 concentrations rose to or above the limit of normal (144 nmol/L) in 12 of 14 subjects in group F2, while the other 2 patients in the group had high FTI values related to consistently higher RT3U. Both the mean values for the study population and for group F2 were statistically higher than baseline from month-one onwards.

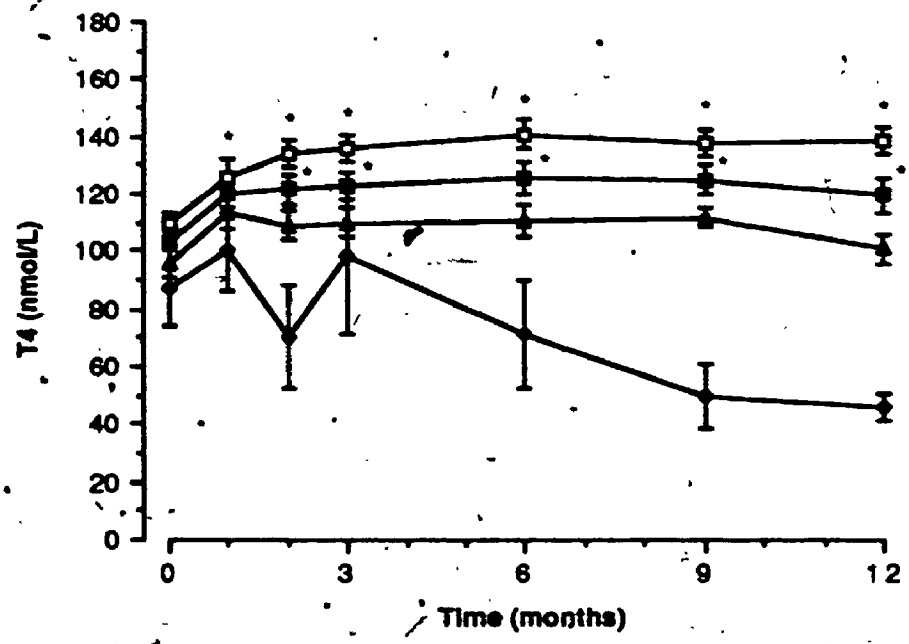

Figure 3-25. Mean thyroxine concentration ± SE for subgroups of patients with and without development of abnormal FTI values on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ■ - Mean values for all subjects available for analysis (n=30).

▲ - Group F1 - subjects with FTI remaining normal (n=13).

□ - Group F2 - subjects developing elevated FTI (n=14).

● - Group F3 - subjects who developed biochemical hypothyroidism (n=3).

* $p < 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

Mean triiodothyronine concentration was never outside the limits of normal in any patient. However, the mean T3 showed a transient statistically significant elevation ($p<0.05$, two way ANOVA, Fisher LSD) during the second month in group F2 followed by a return to baseline. The converse trend toward a transient fall in T3 at 2 months was observed in patients developing hypothyroid indices, as illustrated in Fig. 3-26.

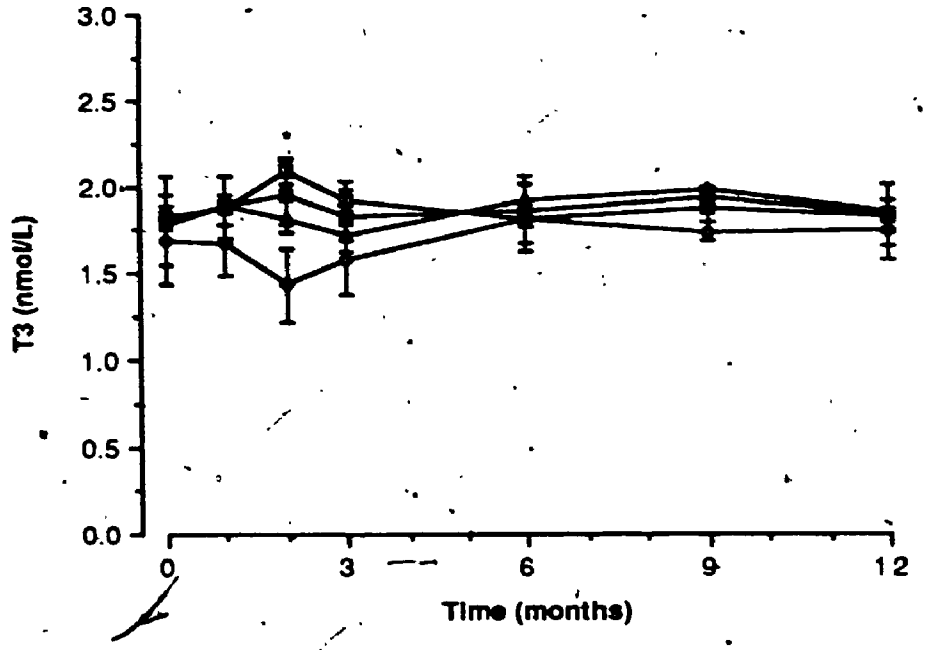

Figure 3-26. Mean triiodothyronine concentration ± SE for subgroups of patients with and without development of abnormal FTI values on amiodarone.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ■ - Mean values for all subjects available for analysis (n=30).

▲ - Group F1 - subjects with FTI remaining normal (n=13).

□ - Group F2 - subjects developing elevated FTI (n=14).

● - Group F3 - subjects who developed biochemical hypothyroidism (n=3).

* $p< 0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

The serum DEA concentrations of groups F1 and F2 were not statistically different during the study. Fig. 3-27. illustrates the relationship between mean T4 and log mean serum DEA concentration. Least squares linear regression of the first four points of the mean concentration-response curve for Group F2 demonstrated a correlation with $r^2 = 0.99$ ($p<0.002$). The T4 concentration did not change in relation to DEA concentration in Group F1.

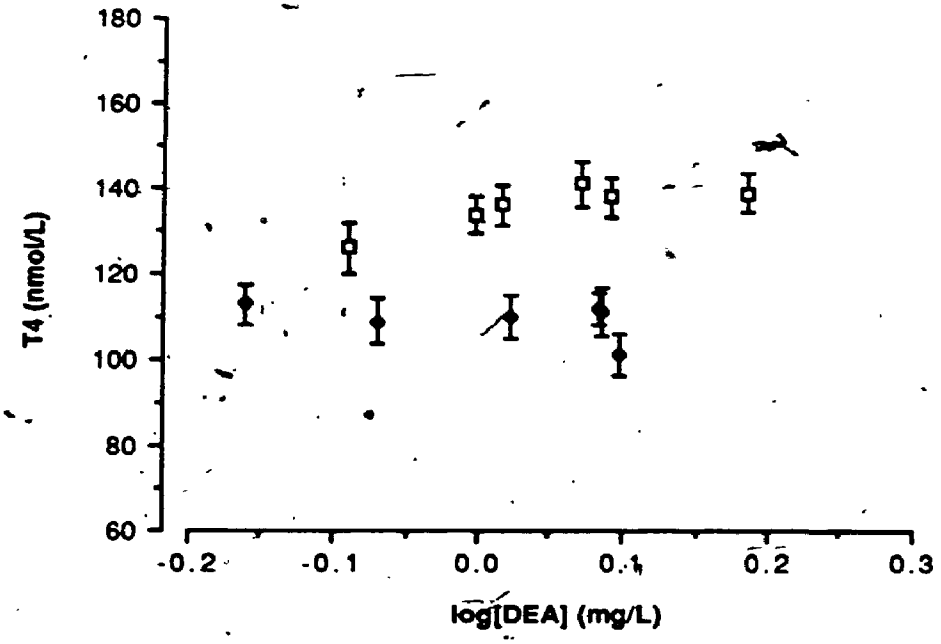

Figure 3-27. Mean thyroxine concentration ± SE vs log of mean [DEA] for subgroups of patients with and without development of abnormal FTI values on amiodarone.
Observations from follow-up visits at 1,2,3,6,9 and 12 months.
Symbol key: ▲ - Group F1 - subjects with FTI remaining normal (n=13).
□ - Group F2 - subjects developing elevated FTI (n=14). Least squares linear regression of the first four points for Group F2 demonstrates a correlation with $r^2 = 0.99$ ($p<0.002$).

The following observations concerning the effects of amiodarone on thyroid function were made in two subjects. One year prior to the study, subject

10 had elevations of T4 during 6 months of treatment with amiodarone. These values returned to baseline 12 weeks after the medication was stopped but once again became elevated above normal while receiving amiodarone as part of the study. Subject 37, who was excluded from analysis because of an elevated T4 at baseline, developed normal thyroid indices of function while taking amiodarone during two weeks of follow-up in hospital.

3.1.9 Other adverse effects

Two patients experienced moderate phototoxicity after ignoring warnings about sun exposure. Three others developed bluish discoloration of the skin near the end of the year of therapy. One had flushing and headache which resolved with time. One developed a vesicular rash on his palms at the time of initiating therapy with amiodarone which resolved within three weeks. One had ecchymoses on the arms which resolved after six weeks with no changes in medication. Platelet count and prothrombin time were normal. One had mild paraesthesia of the left foot which did not progress during continued treatment with amiodarone and one had diarrhea which resolved in two weeks without discontinuing amiodarone therapy.

3.2 Superoxide dismutase activity

SOD activity was measured in 30 patients and 6 healthy volunteers over a period of one year. The healthy volunteers were not selected as a control group but instead were studied as a means of estimating the magnitude and variability of SOD activity in non-patients to aid in interpreting any observed variations in SOD activity. Samples from patient 4 were lost because of organizational difficulties early in the course of the study. The individual data are tabulated in Appendix 5. Figure 3-28 illustrates mean SOD activity for the patient population and volunteers during the study. Baseline values in the patients and the healthy population were statistically different ($p<0.05$, unpaired t-test).

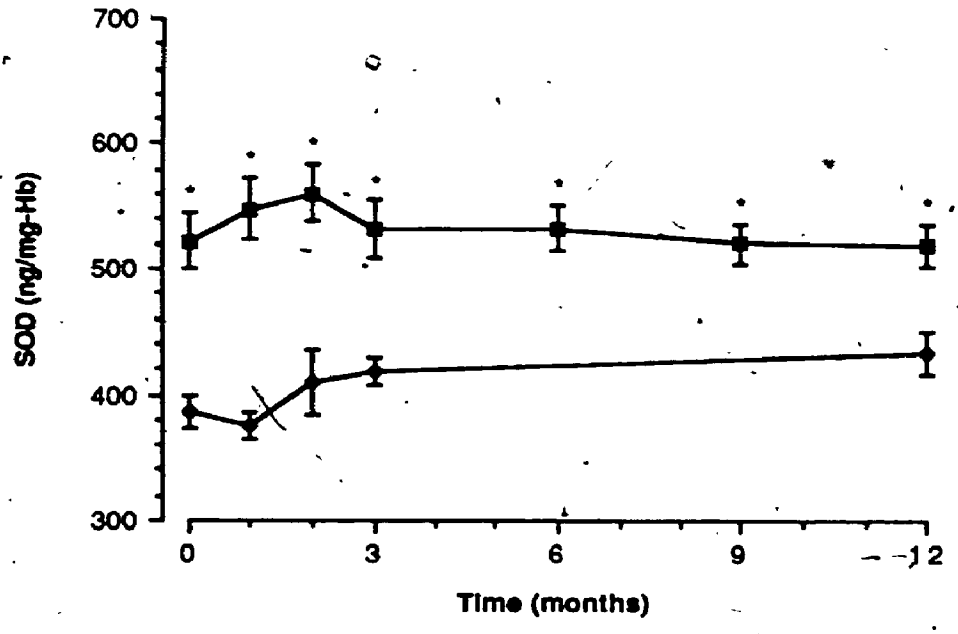

Figure 3-28. Mean SOD activity ± SE for 30 patients on amiodarone compared to the mean ± SE for 6 healthy volunteers.
Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months. Differences in mean erythrocyte SOD activity between cardiac patients and healthy volunteers. Symbol key: ■ - 30 patients taking amiodarone, ◆ - 6 healthy volunteers. Values at all time points are statistically different between groups. * $p<0.05$ (unpaired t-test).

Neither the healthy volunteers nor the patient group as a whole demonstrated statistically significant changes over the year. When those patients who developed subclinical pulmonary toxicity (group D2) were compared to those who did not (group D1), statistically significant differences in SOD activity were apparent. These differences between groups D1 and D2 are illustrated in the two plots of Fig. 3-29. Baseline values for groups D1 and D2 differed, being 493 ± 26.7 ng/mg-Hb and 595 ± 32.4 ng/mg-Hb respectively ($p<0.05$, unpaired t-test). The mean SOD activity for group D1 did not change over the year. In group D2, there was a statistically significant fall from 595 ± 32.4 at baseline to 526 ± 31.3 ng/mg-Hb ($p<0.05$, two way ANOVA) in the first month and a continued falling trend to values reaching a minimum value of 479 ± 24 ng/mg-Hb at nine months. The mean of the 12 month values was higher but not significantly different from the 9 month value. The patient in group D2 who developed overt pulmonary toxicity after the end of the study had a baseline value of SOD activity of 592 ng/mg-Hb which fell to a value of 435 ng/mg-Hb by the end of follow-up.

The other organ frequently involved in free radical toxicity is the liver. Differences between the SOD activities of patients developing hepatotoxicity (group H2) and those who did not (group H1) did not reach statistical significance. Group H2 had a trend towards a negative linear relationship between SOD activity and log serum DEA concentration which was not apparent for group H1 (Fig. 3-30).

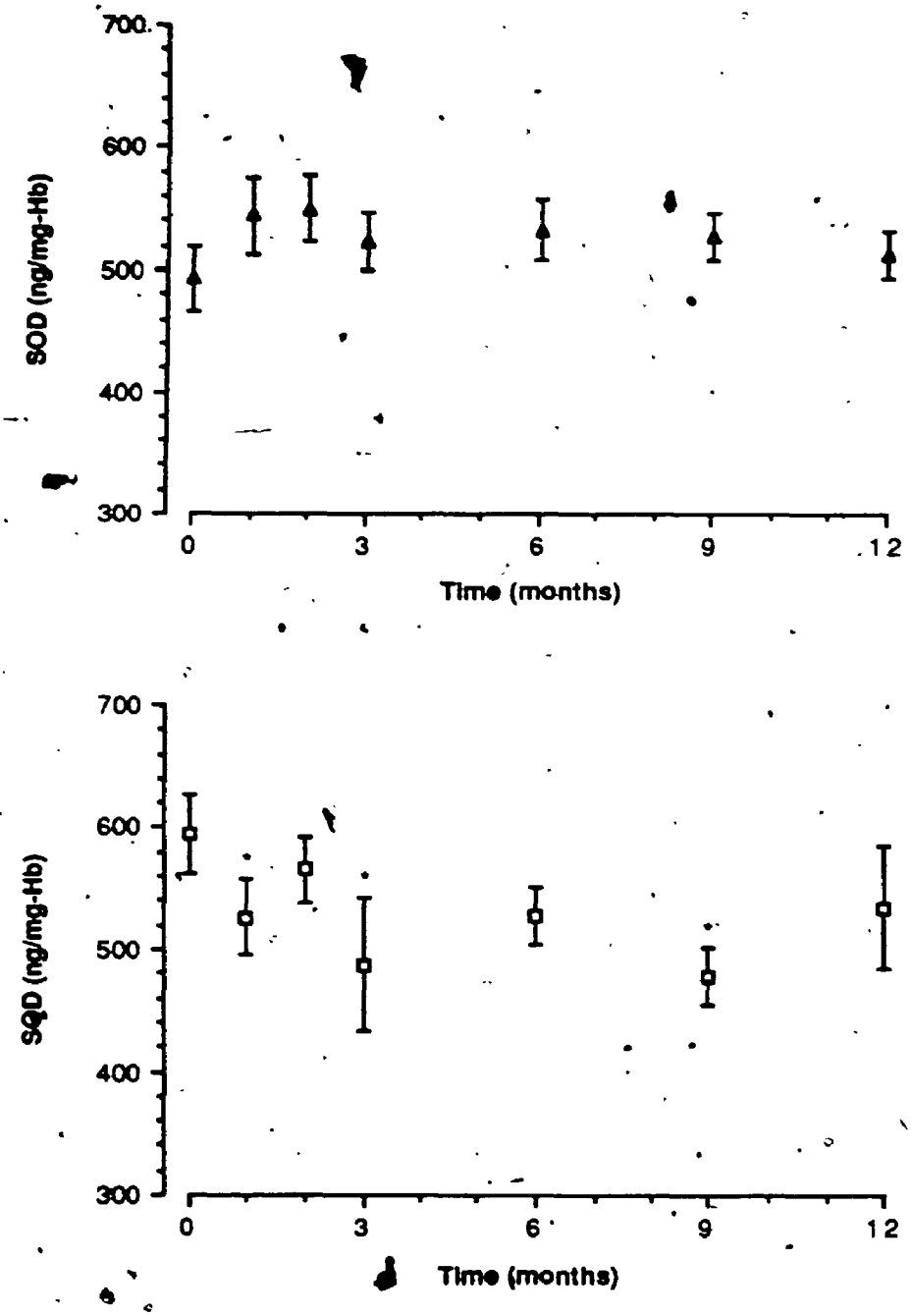

Figure 3-29. Mean SOD activity ± SE for subgroups which did and did not develop subclinical amiodarone pulmonary toxicity.

Observations from baseline and follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ▲ - Group D1 - (above) no pulmonary toxicity (n=24);

□ - Group D2 - (below) pulmonary toxicity (n=6).

*$p<0.05$ difference from baseline (two way ANOVA, Fisher LSD comparison).

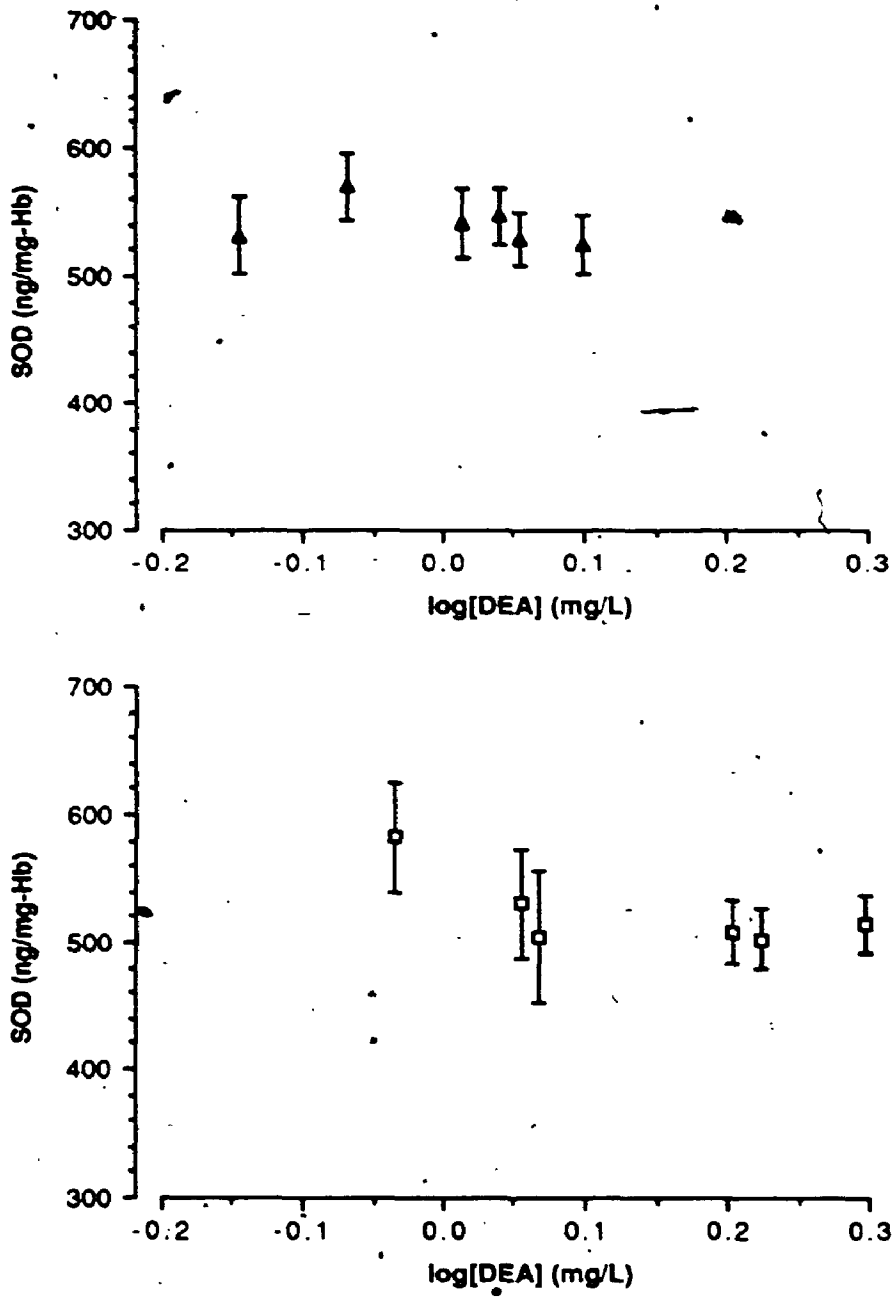

Figure 3-30. Mean SOD activity ± SE vs log [DEA] based for subgroups which did and did not develop subclinical amiodarone hepatotoxicity.

Observations from follow-up visits at 1,2,3,6,9 and 12 months.

Symbol key: ▲ - Group H1 - (above) subjects with no hepatotoxicity (n=22); □ - Group H2 - (below) subjects with hepatotoxicity (n=8). No statistically significant changes in SOD activity occurred in these groups, although a trend to declining SOD activity with increased [DEA] is visible in group H2.

3.3 Summary of adverse effects

The development of corneal microdeposits is an undesirable effect of amiodarone. It is almost always asymptomatic making it difficult to consider a toxic event. Because the occurrence of CMD is virtually universal in patients taking amiodarone, it would be a poor parameter to discriminate differences between patients unless some attempt is made to subclassify the CMD into grades. Toxic effects were sought in three spheres; pulmonary, hepatic and thyroid. Criteria for toxicity were defined for all three categories in the study design. In the case of the changes observed in thyroid function it was known that the iodine load incurred during therapy with amiodarone could not be ruled out as the cause. Thus of the three areas of toxicity, only changes in pulmonary and hepatic function were expected to result from the amiodarone molecule itself and therefore to possible correlate with SOD activity if free radicals were involved in the pathogenesis of amiodarone toxicity.

The adverse effects occurring in the 31 patients studied are summarized in Table 3-4, with the patients ranked by the number of major adverse effects which they developed. Dose administered and concomitant medication for each patient is also listed. Ten of the subjects (32%) had no adverse effects in the pulmonary, hepatic or thyroid categories. Thirteen subjects had changes in pulmonary and/or hepatic function considered to be evidence of toxicity. Seven others had changes in thyroid function alone. None required the termination of therapy with amiodarone. Only the group of patients with changes in pulmonary function showed statistically significant changes in SOD activity. The use of concomitant medications was not different between those with toxicity and those without. Specifically there was no difference in the use of diuretics between the group with rising cholesterol concentrations and the rest of the population (See

Discussion). The incidence of cholesterol elevations and adverse effects in the category "other" tended to cluster with the patients having toxicity in the three major categories. Ranking the average dose shows a statistically significant trend towards higher daily dose per kg with increasing incidence of adverse effects per patient ($p<0.004$ Spearman rank correlation).

Although several patients had more than one adverse effect, the incidence of each separate category can be summarized as follows. Pulmonary carbon monoxide diffusion capacity was abnormal in 6 of 30 patients analysed (20%). Hepatic enzymes were abnormal in 8 of 30 patients analysed (27%). Abnormalities in thyroid function occurred in 17 of 30 patients (56%) with normal baseline function. Ten of 21 subjects (48%) with normal baseline values of serum cholesterol developed elevations above the 75 percentile. Other statistically significant changes observed in the population as a whole were the increase in QTc, decrease in heart rate and increase in serum creatinine concentration.

Table 3-4. Summary of toxic effects observed in 31 patients ranked by number of major effects

Code	Total Adv.Eff.	Age	Sex	Wt. kg.	Average Dose/kg	Dose Rank	Pulmonary	Hepatic	Thyroid	Cholesterol	Other Effects	Other Medication
05	0	68	M	80	2.50	2				NM		N
07	0	36	F	90	3.18	5				NM		
08	0	49	F	82	4.57	16				NM		M
11	0	46	M	68	4.09	11					Phototoxicity	D,H
12	0	68	M	76	2.63	3						H
14	0	65	F	76	3.95	8				Abnormal		H
15	0	72	F	51	7.31	29				Elevated		D,F,N
17	0	66	M	91	4.40	13					Rash	D
22	0	58	F	89	2.25	1				Abnormal	Bruising	H
23	0	25	M	66	6.06	25						N
25	0	40	M	125	3.20	6				Elevated		A
31	0	53	F	115	3.48	7						F
06	1	48	F	120	2.96	4			Hyper	NM		
09	1	56	M	68	4.49	14			Hyper	Abnormal		
10	1	29	M	89	4.49	15			Hyper	Elevated	Phototoxicity	
18	1	52	M	75	3.96	9			Hyper	Elevated	Diarrhea	D,F,N
24	1	68	M	70	5.26	23			Hypo	Elevated		D,H,T4
29	1	65	M	76	5.26	24		NA	Hyper			D,F,H
33	1	63	F	71	6.61	28			Hyper	Abnormal		D
37	1	68	M	78	7.69	30	DCO		NA	Elevated	Paraesthesia	
20	2	50	M	98	4.08	10		ALT	Hyper		Blue skin	H
38	2	51	M	92	4.35	12		ALT	Hyper	Abnormal	Blue skin	D,F,N
21	2	67	M	79	5.06	22		ALT	Hyper			D,F,N
27	2	53	M	68	9.54	31		ALT	Hyper	Elevated		P
28	2	37	M	82	4.71	17		ALT	Hyper		Flushing	
04	2	54	M	81	4.76	18	NM	ALT	Hypo	NM		
36	2	49	M	82	4.88	19	DCO	ALT				A,F
13	2	63	M	80	5.00	21	DCO		Hyper		Flushing	
34	2	75	M	63	6.35	27	DCO		Hyper	Elevated		D
35	2	58	M	70	4.98	20	DCO		Hyper	Elevated		D
19	3	72	M	64	6.25	26	DCO	ALT	Hypo	Elevated	Blue skin	

Code - patient I.D. number
Total Adv.Eff. - total number of adverse effects occurring in pulmonary, hepatic or thyroid categories
Average dose/kg is per day
Dose Rank - from lowest to highest average dose/kg
NA is not analyzed
DCO is pulmonary toxicity
ALT is hepatic toxicity
NM is not measured
D is digoxin, F is furosemide, H is hydrochlorothiazide, A is allopurinol, N is nitrates, M, is metoclopramide, P is propafenone
Abnormal signifies serum cholesterol above 75 percentile at baseline vs. elevated meaning cholesterol became abnormal during the study.

CHAPTER 4 - GENERAL DISCUSSION

4.1 INTRODUCTION

The two goals of this study were: 1) to evaluate the relationship between serum drug concentrations and amiodarone-induced effects; 2) to define better the relationship between administered dose and serum drug concentrations. Achieving the former goal would provide new information linking current knowledge about the kinetic and dynamic behaviour of amiodarone. Such information is important for the assessment of the utility of therapeutic drug monitoring. This need was stressed by Holt et al (1983) at an American Heart Association symposium on amiodarone. Achievement of the latter goal would provide an aid or possibly an alternative to the therapeutic monitoring of serum drug and metabolite concentrations.

The results of this study contribute new information which demonstrates concentration-response relationships for several effects of amiodarone and related concentrations prior to steady state to accumulated dose. The abnormalities which were observed in cholesterol and creatinine concentrations have not been formally documented before in humans. Observation of superoxide dismutase activity in the patients of this study demonstrated that a decrease in SOD activity occurred concurrently with the development of subclinical pulmonary toxicity. One patient with pulmonary dysfunction accompanied by a 27% fall in SOD activity later developed overt pulmonary toxicity. These preliminary findings suggest that a possible contribution of free radicals to the pathogenesis of amiodarone pulmonary toxicity should be considered in future studies.

4.2 INTERPRETATION OF THE RESULTS

The data collected during the course of this study are extensive and therefore the discussion of the results has been divided into subtopics. Preliminary analyses were made of each parameter to identify any changes occurring in the study population as a whole. Where changes in a parameter fulfilled the criteria for toxicity in some patients, further exploratory analyses were carried out to look for differences between the subpopulation which developed the toxicity and that which did not. The process was facilitated by consistently identifying the patients not developing the abnormality as group X1 and those developing abnormalities group X2 (where "X" is an alphabetic character indicating the parameter concerned). Subgroups for pulmonary, hepatic and thyroid toxicity and for cholesterol abnormalities were identified and compared with respect to demographic data, quantity of amiodarone received (mg/kg) and DEA concentrations.

4.2.1 Serum drug concentration

Inspection of the concentration-time curves for serum amiodarone and DEA in individual patients reveals a wide variability in the amiodarone concentration (Appendix 6). A major contributor to interpatient variability is likely the low and variable oral bioavailability of the drug (Holt et al 1983). The intersubject variability of serum amiodarone concentrations is greater than that of DEA and the undulations observed in the serum amiodarone concentration-time curve are not reflected to the same degree in the curve for DEA. The undulations in individual amiodarone concentration curves might result from temporal changes in oral bioavailability or failure of patients to withhold their medication

prior to blood sampling. Averaging individual concentrations produces a mean concentration-time profile for amiodarone which has less undulation than many of the individual curves. Mean amiodarone concentrations rose to 2.5 mg/L by 6 months and were not significantly different from this value during the last six months of the study. Mean DEA concentrations were approximately 0.5 those of amiodarone. The maximum mean concentration of DEA of 1.4 mg/L was reached only at 12 months implying that a plateau was not achieved until sometime after the end of the study.

Serum concentrations of DEA, which has a longer elimination half-life than amiodarone, rise and fall more slowly than those of amiodarone after oral administration of the drug (Kannan et al 1982). DEA concentrations reflect metabolic conversion of parent drug already absorbed, are less susceptible to the effects of acute variations in bioavailability and are less affected by absorption of the most recent amiodarone dose than are the concentrations of the parent drug. For these reasons, the concentration-time profile for DEA may have more utility than that of amiodarone in assessing the overall exposure of the patient to amiodarone.

Prior to steady state, attempts to correlate serum concentrations with daily dose cannot succeed. The long elimination half-life, estimated to be 4 months in this study, predicts that steady-state DEA concentrations would not be attained for 16 to 20 months in patients taking amiodarone. In the current study, which was designed to end before this length of time, accumulating serum drug concentrations were compared to accumulated dose adjusted for body weight, rather than trying to compare a concentration at a single time to daily dose administered to the patient. This approach (Fig. 3-2) demonstrated a strong correlation for mean data (r^2 = 0.98) between serum DEA concentration and

total accumulated dose adjusted for body weight and a similar correlation for amiodarone concentrations ($r^2 = 0.93$).

Falik et al (1987) reported a poor correlation ($r = 0.364$, $r^2 = 0.132$) between individual serum amiodarone concentrations and daily dose of amiodarone, despite having similar findings for concentrations of amiodarone and DEA (2.4 mg/L and 1.8 mg/L respectively) and for DEA:amiodarone ratio (0.6). Their approach illustrates the probable reasons for the failure of many studies to obtain good relationships between serum drug concentration and administered dose. In analysing this relationship, dose administered was not corrected for body weight, steady-state was assumed in patients with a duration of follow-up as low as 2 months and the advantage conferred by the smaller variability in DEA concentrations was overlooked. In studying 111 patients their data revealed that serum DEA concentrations had a at least as good a predictive value (78%) for adverse effects as did amiodarone (76%) and confirmed that DEA concentrations were less variable than those of amiodarone. Stäubli et al (1983) were able to obtain a correlation ($r^2 = 0.62$) between "steady-state" serum amiodarone concentrations and administered dose by correcting dose for body weight. Still better results were obtained in the current study by avoiding the use of "steady-state" comparisons during the first year and adjusting the accumulated dose for body weight.

The strong relationship identified may allow a rough prediction of the serum DEA and amiodarone concentrations of in relation to dose of amiodarone administered during the first year. The weakness of this approach is that while the slope of this relationship may be similar in different patients the intercept may vary, producing different predicted values for serum concentration in relation to accumulated dose.

Several effects of amiodarone were found to be correlated with serum DEA concentrations in this study and are discussed under the appropriate sections. There were prior reasons to suspect that such relationships existed and the correlation of DEA concentration and drug effects is in agreement with the findings of recent pharmacological studies. Mason et al (1985) showed that total accumulated dose of amiodarone was strongly correlated with the incidence of adverse effects in a pooled study of over 1300 patients. Since serum DEA concentration was strongly correlated with accumulated dose in this study, this implied that DEA concentrations should also correlate with adverse effects. Fraser et al (1984) had correlated elevated serum DEA concentrations with a higher incidence of toxicity. The pharmacological activity of DEA has also been demonstrated. Effects on both the heart (Yabek et al 1986) and thyroid function (Venkatesh et al 1986a) have been found. The possibility that the pharmacological activity of DEA may be clinically more important than that of amiodarone has been raised in a recent paper by Nattel et al (1988). They used a canine ventricular tachycardia model to show that the EC_{50} of intravenous amiodarone is 3.2 times greater than that of DEA for suppressing arrhythmias in these dogs. After mg equivalent doses, DEA accumulated to higher concentrations in the heart than did amiodarone. They suggested this preferential concentration of DEA in tissues accounts for some of its greater potency in comparison to amiodarone. DEA also concentrates to a higher degree than amiodarone in the organs which develop the most serious toxicity (lung and liver), reaching levels in excess of 1000 mg/kg of tissue (Holt et al 1983). Adams et al (1985) demonstrated that concentrations of DEA are ten-fold higher in skin biopsies from patients affected by pseudocyanosis than in other patients taking amiodarone.

In previous studies, elimination half-lives have only been estimated in patients who had stopped taking the drug. Elimination half-lives for amiodarone

and DEA estimated in such a way by discontinuing medication and monitoring declining serum drug concentrations have been reported as 53 d and 61 d respectively by Holt et al (1983) and as 47 d and 63 d by Marchiset et al (1985). Estimates of amiodarone half-life as long as 100 d have been cited (Latini et al 1984b). Given an elimination half-life of this length, a patient stopping treatment with amiodarone during a one year study is unlikely to present a good opportunity for observing a decline in serum drug concentrations. Early withdrawal of amiodarone would produce a low starting concentration while later withdrawal would allow only sufficient time to observe the decline in concentrations over one to two half-lives. The approach in the current study allows several concentration-time points (Fig. 3-3) to contribute to the estimate of elimination half-life without stopping the medication in the patient. The half-life of 56 d estimated in this fashion for amiodarone is consistent with previous estimates. The half-life of 129 d estimated for DEA is longer than most previous reports (Marchiset et al 1985). This may reflect the ability of current method to observe changes in concentration for three half-lives of the metabolite and the fact that drug therapy was continued throughout the time when data was collected. None of the patients in this study developed toxicity which merited discontinuation of the medication.

The strong relationship between DEA concentrations and accumulated dose of amiodarone implies that effects of the drug related to DEA concentration will also be related to accumulate dose prior to steady state. Since for many patients, most of their clinical follow-up will be prior to achievement of steady-state DEA concentrations, a record of the accumulated dose may be of use in making therapeutic decisions. Based on an elimination half-life estimated at 4 months, the period prior to steady state for serum DEA (5 half-lives) will extend well into the second year after starting therapy with amiodarone. Because of

this drug's unusually long elimination half-life, daily dosing of amiodarone produces a situation which can be conceptualized as high frequency bolus dosing. A daily dosing interval (24 h) with absorption mainly completed by 5 hours is in contrast to the elimination half-life (1440 h) for amiodarone. Scaling of these times to a model with an elimination half-life of one hour, the corresponding dosing interval would be 1 minute and absorption take place in 12 seconds. Continuous production of the metabolite, DEA, and its longer half-life further modulates swings in its concentration, even more closely approximating a continuous infusion. During the first year of treatment this analogy may be useful to bear in mind when assessing the effects of changing doses. The lesser tendency for undulation and smaller intersubject variability of DEA concentrations provides an advantage over amiodarone concentrations by increasing the power for obtaining correlations with observed responses.

4.2.2 Cardiac effects

The negative chronotropic effects of amiodarone on the heart rate reached a maximum during the first month of therapy (Fig.3-4). *In vitro* studies of activity in rabbit atrial node demonstrate that bradycardia is due to direct actien of amiodarone on sinus node activity with decreased diastolic depolarization. Bradycardia could not be attributed solely to the β-blocking activity of amiodarone which differs from that of propranolol in that it is non-competitive and is unable to completely abolish the effects of β stimulants (Goupil and Lenfant 1976). Radiolabelled ligand binding studies in rat-heart microsomes also show that amiodarone fails to displace β-blockers from receptor sites (Nokin et al 1983). During the current study, effects on sinus node automaticity did not occur in parallel with effects on QTc. In contrast to the concentration-response

relationship observed for QTc, there was no correlation observed between heart rate and serum drug concentrations. This might be explained by an extremely potent effect of amiodarone on heart rate producing a concentration-response relationship for heart rate which reached its maximum before the first measurement of drug concentrations in the current study design. If such a response curve exists, the bradycardic effect must reach a maximum at serum drug concentrations lower than those attained during the first month of this study.

Amiodarone prolongs the action potential duration in cardiac tissues as do other Class 3 antiarrhythmic agents. This effect is observable in electrocardiographic recordings as a prolongation in the QT interval. Finerman et al (1982) followed patients taking 600 to 800 mg of amiodarone daily for an average of 12 weeks and demonstrated a prolongation of the QTc (QT interval corrected for the influence of heart rate) from 294 msec to 494 msec. Baerman et al (1985) were unable to show a correlation between QTc and serum drug concentration with measurements at 1, 3 and 6 months while Debbas et al (1984) have shown a relationship between QTc and myocardial amiodarone concentrations.

In the current study the mean QTc recorded in 31 patients steadily increased over the year of follow-up (Fig 3-5). This implies that the effects of amiodarone on cardiac electrophysiology were increasing throughout this period. The QTc continued to increase in length during the last six months of the study when changes in amiodarone concentrations were less apparent than those of DEA. DEA concentrations, which continued to increase throughout the study, closely paralleled the changes in QTc. This is consistent with the known pharmacological activity of DEA previously demonstrated in dog hearts (Yabek et al 1986, Nattel 1986, Nattel et al 1988). Even if the activity of DEA is weaker than that of amiodarone, accumulations of DEA in myocardial tissue which are 3.6 times that of amiodarone (Marcus 1984) may produce an important contri-

bution to the lengthening of QTc. The data of Nattel et al (1988) in fact would suggest that DEA and amiodarone are equipotent in their cardiac effects when normalized for differences in myocardial drug concentrations. Debbas et al (1984) have advocated the use of QTc as a simple indirect parameter of drug effect and Torres et al (1983) has shown that lengthening of the QTc in patients taking amiodarone is associated with a good prognosis. The results of the current study suggest that increases in QTc length reflect rising DEA concentrations and may be useful to predict serum drug concentrations. Steady state serum DEA concentrations were not reached during the study. A study of QTc extended until steady state would permit observation of whether QTc length stabilizes when DEA concentrations stop rising.

The degree of arrhythmia control observed in the current study (89%) is within the range of efficacy previously reported by other investigators. The lowest efficacies reported, 50% by Waxman et al (1982), 65% by Kaski et al (1981) were from early investigations of the drug in refractory arrhythmias. Withdrawal from amiodarone because of adverse effects lowers its overall efficacy, but a high percent of patients who remain on amiodarone (up to 94%) are successfully treated (Nademanee et al 1983). More recent studies with less refractory patients have reported better success rates, for example 82% by Falik et al (1987). In the current study only 3 subjects did not achieve control with amiodarone alone within 6 months of starting. Control was achieved before the maximum effects on QTc. Unfortunately good results in the first year of therapy do not appear to extend into the long-term. Smith et al (1986) calculated that the actuarial probability of remaining successfully treated on the drug at 50 months was only 19%. Such a decline in efficacy is likely attributable to a combination deteriorating patient health in a high risk population and the

increasing rate of withdrawal from amiodarone because of adverse effects as duration of therapy increases.

4.2.3 Corneal effects

The reported 98% incidence of CMD and the lack of evidence of permanent ocular damage (Ingram 1983) have lead most investigators not to report CMD as toxic events. In the current study CMD (corneal microdeposits) were considered to be an observable effect of amiodarone therapy rather than toxicity. Several investigators state that the development of CMD is related to the dose and duration of amiodarone therapy without specifically defining this relationship (D'Amico et al 1981, Nielsen et al 1982 and Ingram 1983). Stäubli et al (1983) found a poor correlation between steady-state amiodarone concentration and grade of microdeposits, while Kaplan and Cappaert (1982) showed a graded progression of CMD in relation to total accumulated dose of amiodarone.

In the current study 84% of patients had CMD at 3 months of follow-up. This is comparable to the 94% found by Orlando et al (1984). Development of CMD in the current study was progressive over time and plateaued between grade 2 and 3 in most patients. Microdeposit grade progressed in parallel with increases in serum DEA concentrations. The mean grade of CMD was 1.66 at the sixth month of follow-up. The median time for CMD development of 6 months predicted from life table analysis by Biron et al (1987) corresponds to the 50% incidence of grade 1 CMD at 6 months observed in the current study.

One subject experienced visual disturbances because of CMD in the current study. This was limited to seeing halos around bright lights at night. One subject reverted from trace CMD to clear corneas at 9 months of follow-up,

corresponding to a fall in DEA concentrations from 1.23 mg/L to 0.68 mg/L. Poor compliance with the therapeutic regimen was considered the reason for the falling DEA concentration. Patients who do not develop CMD should have serum drug concentrations measured to verify probable low values if there is a question of inadequate therapy with amiodarone.

In Fig.3-9 a sigmoidal mean concentration-response relationship was demonstrated between the grade of CMD and the log of the DEA concentration. Over the linear segment of this curve between 0.93 mg/L and 1.3 mg/L, CMD were strongly correlated with serum DEA concentration. Grading of CMD, which are easily observable with a slit lamp, can be done by an experienced physician in the clinical setting. This may provide a rapid method for estimating the degree of exposure to amiodarone. DEA concentration is strongly related to both total accumulated dose and the degree of CMD development. A prospective study is required to determine whether measurement of CMD grade provides a simple clinically measurable predictor of the risk for other adverse events. The opportunity to observe the regression of CMD compared to the rate of decline in DEA concentrations in patients withdrawn from amiodarone therapy would also help to confirm or refute this correlation.

4.2.4 Pulmonary effects

Pulmonary toxicity is the most important adverse effect of amiodarone in terms of patient safety. The weighted mean incidence of amiodarone pulmonary toxicity in six studies done during 1982 and 1983 was 5.2 % (Table 1-2). Based on this incidence, the current study of 31 patients had a statistical power of 81 % to observe at least one case of overt pulmonary toxicity. This

power would be even higher if the incidences was considered to be above 10 % as reported more recently by Mason et al (1985) and Magro et al (1985). Six subjects (20%) of the 30 followed with pulmonary function testing developed progressive falls in DCO (diffusion capacity) which were sustained below 80% of baseline for the duration of the study. These patients were thus classified as having subclinical pulmonary toxicity as defined in the study design. One patient with subclinical toxicity developed overt pulmonary toxicity ten months after completing the study. These findings are comparable to those of Anastasiou-Nana et al (1985) in which one third of the patients receiving amiodarone developed a 20% decrease in DCO and one third of these proceeded to develop overt pulmonary toxicity. The findings also concur with the statement by Magro et al (1985) that DCO is the best test of pulmonary function for predicting the development of pulmonary toxicity. As with the current study, they also did not find any difference in baseline DCO between patients developing toxicity and those not.

There is a wide disparity in the incidence of pulmonary toxicity reported in the literature. No cases were reported during the first 15 years of experience with this drug in Europe. A recent study of 119 patients in Britain designed to identify this complication failed to observe any occurrences of pulmonary toxicity (Finnegan and Faragher, 1985) as was the case in 53 patients studied in Scandinavia (Emmertsen et al, 1987). This contrasts with a 15% incidence reported from a study in Texas (Magro et al 1985) and a 13% incidence reported from Utah (Mason et al 1985). Some of these differences may be explained by differences in the health of the patients entered in these studies. Differences in sensitivity of detection seem unlikely to explain the negative findings of investigators such as Finnegan and Faragher (1985). The possibility of genetic differences in the metabolism of amiodarone or other metabolic func-

tions was raised by Harris et al (1983c). The discrepancy in incidence is so large that this question cannot be discounted especially in view of the geographic differences between the populations studied in these conflicting reports.

Comparisons were made between the six patients developing subclinical toxicity (group D2) and those who did not (group D1). Baseline DCO did not differentiate the groups. Group D2 had a progressive statistically significant decrease in DCO, while the other patients had only small variations around their baseline value. Differences between groups could not be explained on the basis of age, weight, dose administered or serum DEA concentrations. The only two smokers in the study were both in the group D1. There was no response to rising DEA concentrations in group D1, while a concentration-response curve existed for the mean fall in DCO vs log DEA concentration in group D2 (Fig 3-11). This suggests differences between groups in their susceptibility to the pulmonary effects of amiodarone.

4.2.5 Hepatic effects

Transient elevations in serum hepatic enzymes are common in patients taking amiodarone (Haffajee et al 1983). Criteria for hepatotoxicity in the current study (doubling or more in concentration of both serum hepatic transaminases) proved to a be a good discriminator for patients developing elevations of hepatic enzymes which were sustained for several months. Whereas patients developing prolonged subclinical hepatitis had parallel elevations of both transaminases, two patients developing concentrations twice baseline of only one transaminase hepatic enzyme, proved to have only transient elevations of these enzymes. Rigas et al (1986) found similar parallel rises in serum hepatic transaminases in patients with hepatotoxicity.

Serum alanine transaminase concentration (ALT) was statistically elevated over baseline in the population as a whole (n=30) from the sixth month onwards in the current study. Most of the rise in ALT occurred in those patients with subclinical hepatotoxicity (H2) in whom ALT continued to rise throughout the year. Group H1 developed only small elevations in ALT concentration over the course of the year. Harris et al (1983b) showed a linear relationship between log AST concentration and DEA concentration with an $r = 0.62$. In the current study, the log DEA concentration-effect relationship for changes in ALT (Fig. 3-14) had a correlation coefficient for mean data of the population as a whole of $r = 0.92$ ($r^2 = 0.85$). The mean DEA concentrations of group H2 were statistically higher than those of group H1 from six months onwards. There appears to be a difference in the response of the two groups to a given concentration of DEA. The ALT in Group H1 did not change in relation to changes in DEA concentration whereas ALT was strongly correlated to DEA concentration in group H2. Differences between the groups could not be accounted for on the basis of age, weight or mean dose/kg. Group H2 did have abnormal values of ALT at baseline which suggests that prior hepatic abnormalities may predispose to the development of subclinical hepatitis.

4.2.6 Metabolic effects

A statistically significant rise in the mean total serum cholesterol was demonstrated in the 26 patients being followed with serum lipid measurements (Fig.3-15). This was accompanied by a statistically significant rise in triglycerides at one and two months. The transient increase observed in triglyceride concentration is similar to that seen in patients after initiating therapy with thiazide diuretics (Burris and Freis 1985). Neither introduction of diuretics nor

changes in diet could readily explain the changes observed in the current study. No patient started diuretic therapy during the study and diuretics were being used by less than half the patients. Diet in hospital was not restricted, baseline measurements were made soon after admission to hospital before any effect of diet at home would have disappeared and only one of the patients was instructed regarding any special dietary prohibitions.

Twenty-one patients were found to have a normal serum cholesterol (below the 75 percentile) for their age and sex at baseline. These patients also demonstrated a sustained statistically significant elevation in serum cholesterol over the year. It is highly unlikely that such a consistent elevation, in a population with normal initial cholesterol concentrations, resulted from random fluctuations. Non-fasting serum cholesterol concentrations have been shown to be similar to those in a fasting state (Schaefer and Levy 1984). Thus small amounts of food eaten by some patients 4 to 5 hours before the blood sampling would have minimal effects on serum total cholesterol concentrations. The effects of food on triglyceride concentrations would increase variability in the data. This higher type II statistical error did not obscure the statistically significant rise in triglycerides observed at one and two months in the 26 patients.

Patients with normal baseline serum cholesterol who developed abnormal cholesterol concentrations (> 75 percentile for age/sex, labelled C1b) were compared with those who did not (C1a). This latter group (n=11) had cholesterol concentrations which differed from baseline only at the second month. Group C1b (n=10) developed a sustained statistically significant elevation in total serum cholesterol concentration, accounted for most of the higher serum triglyceride concentrations seen in the population as a whole and had higher glucose and DEA concentrations than group C1a. The average daily amiodarone dose/kg and serum DEA concentrations during the first 6 months were

also higher in the group developing abnormal cholesterol concentrations. Unlike group C1a where serum cholesterol concentration showed no relationship to serum DEA concentration, the serum cholesterol concentrations in group C1b rose in relation to DEA concentration until the second month. The two groups could not be distinguished from each other on the basis of age, sex, weight or initial cholesterol concentration. Differences between groups also could not be explained on the basis of thyroid status, abnormalities of liver function, concomitant medications or diet. Only 2 of the 10 patients in group C1b developed thyroxine concentrations below 50 nmol/L and this change did not appear until several months after the rise in cholesterol had occurred. As normally seen in patients taking amiodarone (Nademanee et al 1986), all other patients in both groups had some degree of elevation in thyroxine concentration (an average of 15% above initial values). This change would be expected to reduce rather than elevate total cholesterol concentration. Two patients in group C1b had elevations of serum hepatic enzymes while 4 patients in group C1a had such changes. One of the patients in group C1b was receiving a thiazide and 2 received furosemide while 4 patients in group C1a received thiazides and 4 were taking furosemide. These diuretics were all started prior to commencing amiodarone therapy. Differences between these two groups suggest that it is possible that patients with normal baseline serum cholesterol fall into two distinct categories in relationship to the way they metabolize lipids and carbohydrates in the presence of amiodarone.

The five patients with abnormal baseline serum cholesterol concentrations had no statistical changes in their metabolic parameters. One of these patients with a baseline serum cholesterol value of 7.4 mmol/L (286 mg/dL, > 95th percentile) experienced a further increase in cholesterol to 9.2 mmol/L (356 mg/dL). A sustained fall in cholesterol occurred only in the one patient with

abnormal baseline cholesterol who was placed on a weight reducing diet at the time of entry into the study.

The effect of certain cardiovascular medications on serum cholesterol and triglyceride concentrations has been a subject of increasing concern over the last decade. Examples include hydrochlorothiazide, which has been shown to increase low density lipoprotein (LDL) cholesterol and lower high density lipoprotein (HDL) cholesterol as well as having a hyperglycemic effect (Chobanian 1987, Ames 1986b) and several beta-blocking agents which have been shown to decrease HDL cholesterol (Chobanian 1987, Ames 1986a). The true impact of these changes on the overall risk to the patient remains to be determined. Based on the conclusions of the Lipid Research Clinics trial (1984a and 1984b) that a 1% reduction in serum cholesterol is associated with a 2% reduction in cardiac risk, drug induced increases in serum cholesterol may prove to have an adverse affect on atherogenic risk. Therefore small changes in cholesterol concentrations may prove to be clinically important.

Investigations in animal models prior to this study suggested that amiodarone is also a drug which adversely affects serum lipid concentrations. Elevations in triglyceride and total cholesterol concentrations were demonstrated in rabbits (Kannan et al 1982) which concurred with data from internal drug company toxicity studies showing cholesterol elevations in four species of animals (Cordarone® extended product monograph, Ayerst Laboratories, Montreal, Canada). Prior to the current study there were also three isolated reports of amiodarone effects on lipid and carbohydrate metabolism in humans which normalized after withdrawal of the medication (Esterhuysen et al 1983, Politi et al 1984, Pollak and Sami 1984). The results of the current study confirm these anecdotal observations.

This first statistically proven observation that amiodarone alters lipid metabolism does not indicate the mechanism for this effect. Nonetheless, elevations of cholesterol by whatever mechanism should raise concern over the potential increase in atherogenic risk. In future prospective trials with amiodarone the cost of serum lipoprotein determination would be justifiable to document the distribution of cholesterol, in addition to monitoring fasting serum cholesterol, triglycerides and glucose concentrations. Since this study was completed, data has been presented showing that amiodarone apolipoprotein B and increases total cholesterol at the sixth week after commencing therapy suggesting that the low density lipoprotein fraction is responsible for the cholesterol elevation (Kasim et al 1987).

The clinical relevance of these observations remains to be determined. Although many patients taking amiodarone suffer from advanced heart disease or are at such high risk of sudden death that the potential risk of elevated cholesterol may not affect their outcome, the use of amiodarone in younger patients with refractory arrhythmias is becoming more common (Kannan et al 1987). In these patients, an increase in serum cholesterol in the order of 20%, implies a possible 40% increase in atherogenic risk over their lifetime. Age, sex and pretreatment cholesterol concentration did not predict the development of metabolic abnormalities. Since abnormalities were already apparent by the second month, it may be possible to predict the effect of amiodarone on serum cholesterol concentrations as early as 4 to 8 weeks after starting therapy.

4.2.7 Renal effects

The mean serum creatinine in the patients of the current study rose steadily over the period of a year suggesting a 10% decline in renal function.

There was no evidence of electrolyte abnormalities in the study population. There is one anecdotal report of a patient with an unexplained elevation of serum creatinine which varied according to the dose of amiodarone (McGovern et al 1983).

The statistical change in creatinine found in the current study, while not clinically important, poses a question of etiology. Possible causes include a drug related decrease in cardiac output reducing renal perfusion, a drug effect directly on the kidneys, or a general deterioration in cardiac function secondary to the primary heart disease in these patients. A negative inotropic effect of amiodarone is the most unlikely possibility in view of data from DePaola et al (1987) who studied 126 patients using radionuclide estimates of ejection fraction before starting amiodarone and 9 months later. They found no change in the majority of patients while 22 improved their ejection fraction by more than 5% and only 10 worsened by more than 5%. Future studies might be undertaken to measure other parameters of renal function such as renal blood flow to try to rule out direct toxic effect of amiodarone on renal function.

4.2.8 Thyroid effects

Free thyroxine index (FTI), an estimate of free serum T4, proved to be a more sensitive parameter for detecting patients with changes in thyroid biochemistry than was total serum T4. Only two subjects developing abnormal FTI did not have abnormal T4 values. Instead these two patients had high RT3U values implying a higher than normal fraction of free T4. Virtually all the patients studied developed some degree of elevation in FTI and total T4 over the year. None of the patients developed either clinical or biochemical hyperthyroidism. These results parallel those of Nademanee et al (1986) who found

a rise from a baseline T4 of 103 nmol/L (8 µg/dL) to 154 nmol/L (12 µg/dL) in the first three months of therapy, followed by sustained elevations at this level. No changes in T3 concentrations were observed. In the current study, none of the patients had any T3 concentrations outside the limits of normal. Of the 27 patients not developing hypothyroidism, 14 developed biochemical abnormalities with FTI above the upper limits of normal. Rather than diagnose such a large fraction of patients as toxic Nademanee et al (1986) suggested that values for T4 concentrations up to 244 nmol/L be accepted as the upper limit of normal in patients receiving amiodarone. They suggested the lower limit of normal should be raised to 64 nmol/L. Patients with biochemical indices remaining within these limits and not having clinical symptoms of thyroid disease seldom require treatment for these thyroid abnormalities.

The incidence of clinical thyroid disease in patients taking amiodarone is not large, ranging from 1 to 5 % for hyperthyroidism (Marcus et al 1981, Harris et al 1983b) and 1 to 8 % for hypothyroidism (Singh and Nademanee 1983, Nademanee et al 1986). Clinical hypothyroidism occurred in only one patient in the current study. Many patients who develop clinical symptoms may have pre-existing thyroid disease aggravated by the iodine load from amiodarone (Jonckheer 1978, Fradkin and Wolff 1983). There was no evidence of such disease at baseline in any of the patients analysed in the current study.

In the current study, the group (F2) developing biochemical hyperthyroidism (FTI greater than 0.56) had a statistically significant elevation of mean T4 concentration by the 3rd month. All patients in this group developed FTI values greater than 0.60. A trend towards a rise in T4 at the second month in F2 was not seen in F1. Differences between the groups could not be explained on the basis of age, sex, weight or dose/kg or serum drug concentrations. Different responses to serum DEA concentrations were observed in groups F1 and F2.

Group F1 displayed no response to increasing DEA concentrations. Group F2 had a linear rise in T4 concentration in relation to log DEA concentration over a range of 0.77 mg/L to 1.23 mg/L (Fig. 3-27).

The patient excluded from analysis had hyperthyroid indices at the time of entry into the study. These indices normalized within one month of starting amiodarone therapy. The augmented dosing of amiodarone during initiation of therapy may have supplied an iodine load similar to that provided by administering Lugol's iodine, a recognized therapy for hyperthyroidism. Elevated T4 concentrations returned to normal after 3 months in one patient who stopped amiodarone in order to undergo surgery. After entry into the study T4 concentrations were once again elevated. This pattern is consistent with the longitudinal changes of thyroid function in patients receiving amiodarone reported by Singh and Nademanee (1983).

A recent report suggests that amiodarone is thyrotoxic in some patients. Biopsy material in cases of amiodarone-induced hyperthyroidism showed follicular breakdown with the release of thyroid hormones and triiodothyronines (Smyrk et al 1987). This is consistent with a direct toxic effect of amiodarone on membranes. The resultant exposure of thyroid antigens to immunologically active cells may account for the increase in antimicrosomal antibodies in 6 of 10 patients reported by Rabinowe et al (1986).

4.2.9 Other effects

The incidence of gastrointestinal disturbances, skin changes and neuropathy was too low to make any statistical correlations.

4.3 INTERPRETATION OF SUPEROXIDE DISMUTASE FINDINGS

During the design of the trial it was recognized that the measurement of SOD (superoxide dismutase) in erythrocytes does not necessarily reflect the activity of SOD in tissues which develop toxicity. There are few data on normal values of SOD activity in erythrocytes. Tissue levels were estimated at 490 ng/mg for liver and 254 ng/mg for lung by Marklund (1980). Like other tissues of the body, amiodarone enters erythrocytes (Somani et al 1985a-b) and they are affected by the drug, exhibiting the formation of similar lamellar inclusion bodies (Somani et al 1986). If these inclusion bodies reflect the pathologic process which results in amiodarone toxicity and this process involves free radicals, it would be reasonable to expect changes in free radical defenses to occur in erythrocytes as well as other tissues. The advantage of sampling erythrocytes is that they constitute a standard tissue which is conveniently obtained and do not present problems with ethical considerations for investigative work. Erythrocytes would be an appropriate sample tissue if screening the SOD activity of prospective amiodarone recipients was shown to have utility in predicting toxicity.

The mean SOD activity in a group of 6 healthy volunteers aged 29.7 ± 4.3 years, was statistically lower than that of the mean activity for the study population. Gambert et al (1985) have shown an increase in SOD activity with age. This may explain the different mean SOD activity of the study population aged 55.2 ± 2.4 years. There were no statistical differences in age between the subgroups of patients with and without various adverse effects.

Six of the 30 patients followed with DCO (single breath carbon monoxide diffusion capacity) developed subclinical pulmonary toxicity. One of these

patients developed overt pulmonary toxicity after completing the study. There was no change in SOD activity in the population group or in the healthy volunteers. In comparison, the group with pulmonary toxicity had a statistically significant decline in SOD activity while the patient later developing overt pulmonary toxicity had a 27% decline in SOD activity over the course of the study. Another group of patients with subclinical hepatotoxicity showed a tendency to decrease SOD activity which did not reach the level of statistical significance. The fall in SOD activity observed in erythrocytes of patients developing pulmonary toxicity gives rise to the question of why, if not a drug related event, did this fall occur. This question merits further investigation into the effect of amiodarone on the metabolism of free radicals in patients taking this drug.

Amiodarone hydrochloride itself is not a free radical in the form given to patients. Nevertheless, evidence does exist that amiodarone or its metabolites may be activated to a free radical state or in some other way produce tissue injury which appears to be free radical mediated. Amiodarone demonstrates a Type I mechanism of phototoxicity in electron spin trapping (ESR) studies, suggesting it can form a free radical when acted upon by high energy photons (Guerciolini et al 1984). Two primary mechanisms of phototoxicity were described by Cannistraro (1977 & 1982). In both cases the phototoxic agent is elevated to an excited state by the absorption of a photon. In the Type I pathway the activated agent directly interacts with biologic molecules implying free radical activation of the agent (Type II occurs when the activated agent reverses the spin of molecular oxygen, causing the formation of activated oxygen species). Diffey et al (1984) showed a photochemical change in amiodarone when exposed to light of 360 nm wavelength and concluded this photoproduct was the agent toxic to the skin. Hasan and Kochevar (1984) have shown that

the di-desethyl metabolite of amiodarone is a three-fold more potent photohemolytic agent than its parent compound.

The metabolism of amiodarone to reactive furan intermediates by the pulmonary epithelium has been suggested as a mechanism of toxicity (van Zandwijk et al 1983). Direct toxicity has been demonstrated by infusing 10 mg of amiodarone into the inflow circuit of an isolated perfused rabbit lung preparation which produces immediate increases in microvascular permeability and pulmonary edema (Gordon et al 1985) as occurs in pulmonary oxygen toxicity (Crapo and Tierney 1979). Further evidence provided by Martin and Howard (1985) shows that addition of 10-20 µg/ml of amiodarone to cultures of bovine pulmonary artery endothelial cells induces the formation of intracytoplasmic lamellar inclusions followed by increased release of ^{51}Cr indicating direct cytotoxicity. Yap et al (1987) produced lamellar inclusion bodies in the cytoplasm of human hepatocyte cultures in less than 2 weeks of incubation with 10 µg/ml of amiodarone. Gordon et al (1985) found that toxicity in their rabbit lung model could be markedly attenuated by decreasing oxygen concentration, or by addition of N-acetylcysteine, vitamin E or butylated hydroxyanisol. They concluded that amiodarone caused oxidant damage to the lungs. If amiodarone is activated to toxic intermediates in various tissues of the body, adverse effects might then occur in those patients where the rate of production of these intermediates exceeded their genetic ability to detoxify them.

The contribution of free radicals to the toxicity of many drugs is becoming more widely recognized. Many such drug reactions have elements in common with amiodarone toxicity. Common drugs which are metabolized to reactive intermediates capable of causing hepatic and pulmonary toxicity include acetaminophen, nitrofurantoin, halothane and bleomycin (Thrush et al 1982). Bleomycin pulmonary toxicity produces lamellar ultrastructural abnormalities in

the lungs very similar to those seen in amiodarone toxicity (Holoye et al 1978). Nitrofurantoin, which like amiodarone contains a furan ring, is also neurotoxic and pneumotoxic (Thrush et al 1982). Methylphenyltetrahydropyridine (MPTP) produces free radical intermediates which cause neurotoxicity (Javitch et al 1985). One substance abuser rapidly developed severe symptoms of Parkinson's Disease and died after injecting the products of a bad batch of a meperidine analogue (MPPP) containing MPTP. At autopsy neuromelanin pigments (lipofuscins) were seen in the substantia nigra (Davis et al 1979). Amiodarone also causes neuronal lipofuscin deposition (Meier et al 1979).

Ethanol is probably the most commonly ingested substance capable of disrupting liver function. Its intermediate species, acetaldehyde, is further metabolized by xanthine oxidase, releasing $O_2^{\cdot-}$ which may be responsible for its hepatotoxicity (Lewis and Paton 1982). The free radicals generated lead to lipid peroxidation as demonstrated by the accumulation of the 9, 11-linoleic acid isomer in chronic alcoholics. This diene-conjugated fatty acid is a marker of free radical peroxidation of lipids. Its concentration decreases rapidly when alcohol is withdrawn from these patients. The tendency of some alcoholics to develop fibrosing liver damage appears to be determined by the pattern of P-450 oxidation and by other enzymes which can produce free radicals during metabolism of xenobiotics (Fink et al 1985). The findings of alcoholic cirrhosis include ultrastructural abnormalities (including lipofuscin) in the liver which in some cases are indistinguishable from amiodarone-induced hepatotoxicity (Simon et al 1984).

It was recognized that improvements could be made in the methods used to observe changes in free radical scavenging activity. The intrasubject variability of SOD activity in healthy volunteers ranged from 6 to 14%. There was no information on the variability of SOD activity in cardiac patients not taking

amiodarone. Therefore it is difficult to determine to what degree the changes in SOD activity in patients taking amiodarone may be masked by normal variability. A statistical difference was still demonstrated between patients taking amiodarone who developed pulmonary toxicity and those who did not.

The coefficient of variation of the assay for SOD activity was 10% which accounts for most of the variability seen in healthy volunteers. Problems with the current SOD assay stem from the two part methodology where the sample must first be measured for hemoglobin concentration and then assayed for SOD activity. Both these phases were carried out on manually operated laboratory equipment and were subject to CVs of approximately 5% each, producing an overall CV in the order of 10%. Although this CV is comparable to many laboratory tests and much better than some clinically accepted tests such as the glucose tolerance test (Ole[illegible]y and Reaven 1974), reducing this variability would be highly desirable for future studies. The degree of beta-type statistical error resulting from the variability of the current assay may be such that true differences in SOD activity could have been overlooked in the current study. A decreased coefficient of variation for the assay might allow the demonstration of statistical significance for the falling trend in SOD activity seen in patients with hepatotoxicity. Efforts should be made to improve the SOD assay for use in future studies.

4.4 CONCLUSIONS

4.4.1 Relevance to clinical therapy

This study was based on two hypothesis which bear on the clinical management of patients taking amiodarone. The first, that relationships exist between serum amiodarone concentration and the effects of this drug and between the dose of drug administered and serum drug concentration, and the second, that similar relationships exist for serum concentrations of desethyl-amiodarone, are both consistent with the evidence produced by this study.

New information was contributed demonstrating a relationships between serum concentration of the desethyl metabolite and the following effects: 1) lengthening of the corrected QT interval, 2) the development of corneal microdeposits, 3) elevation of serum alanine aminotransferase concentrations and 4) elevation of serum creatinine concentrations. A subpopulation of patients developing pulmonary toxicity had decreases in pulmonary diffusing capacity which correlated with serum DEA concentrations. A subgroup of patients developing free thyroxine indices outside the range of normal had change in serum thyroxine concentration which correlated with serum DEA concentrations. Similar relationships existed for amiodarone concentrations which were generally related to DEA concentrations by a factor of two times, but these relationships were less apparent because of the higher intersubject variability and undulations in the amiodarone concentration profiles.

The relationships documented for DEA support previous work suggesting that the effects of amiodarone correspond not only to the activity of the parent drug but also to that of its metabolite, DEA. The laboratory work of Nattel et al (1988) demonstrated that DEA has effects on the heart similar to those of amio-

darone. Correlation of DEA concentrations with some of the adverse effects of amiodarone suggests that DEA also contributes to the development of toxicity. Thus future studies of adverse effects should consider the contributions of both amiodarone and it active metabolite to the evolution of these effects.

This study demonstrated, for the first time, that the administered dose of amiodarone is related to serum drug concentrations. Because of the long delay in attaining steady state, to be clinically useful, serum drug concentrations must be considered to be representative of accumulating drug in the body for at least the first year after starting therapy. The strong relationship existing between serum DEA concentration and accumulated dose/kg is consistent with such an assumption. The elimination half-lives observed in this study would make the time to attain steady-state concentrations after five half-lives at least 10 months for amiodarone and 20 months for DEA. For this reason attempts to correlate daily drug dose with "steady-state" concentrations before one year of therapy have met with little success.

The correlations between serum DEA concentration and accumulated dose, QTc and corneal microdeposits may be clinically important. Such interrelationships imply that monitoring of these three clinically observable parameters may complement therapeutic drug monitoring in many patients.

In addition to the major objectives of this study, observation of superoxide dismutase demonstrated changes in SOD activity which correlated with the development of pulmonary dysfunction. This is not proof of a free radical mechanism for amiodarone pulmonary toxicity, but the possibility cannot be discounted offhand in view of the current results and the evidence in the literature for free radical activation of amiodarone and its metabolites. The recognition of a role of free radicals in amiodarone toxicity, if it exits, would be clinically important for two reasons. First, it may be possible to develop methods to iden-

tify patients at risk of free radical induced toxicity. Second, therapeutic maneuvers such as decreasing inspired oxygen concentration and the use of pharmacologic free radical scavengers might be used to modify the evolution of toxic effects.

4.4.2 Proposals for further studies

The findings of this study point to several areas where further investigation may prove fruitful. Future research suggested by these results include:

1) a prospective study to confirm the utility of using accumulated drug dose, QTc and grade of corneal microdeposits in predicting serum drug concentrations.

2) a prospective study to confirm the utility of using accumulated drug dose, QTc and grade of corneal microdeposits in predicting changes in serum transaminase concentration, serum thyroxine concentration, and pulmonary diffusion capacity.

3) a prospective study of the effects of amiodarone on serum lipids in which data should be collected frequently enough to detect the time course of changes in serum lipoproteins.

4) a prospective study of the effects of amiodarone on renal function.

5) a study of the relationship between serum drug concentration and the development of bradycardia during the first month of therapy.

6) a population study to explore the relationship between interindividual differences in SOD activity and differences in hepatic metabolism.

7) a study of other parameters indicative of free radical activity in patients to confirm the changes observed in SOD activity in patients with pulmonary dysfunction.

8) animal and/or tissue culture studies looking for evidence of free radical activation of amiodarone and its metabolites with techniques such as whole cell electron spin resonance.

The methodology for the clinical studies would be relatively straight forward. The inclusion of a control group of cardiac patients would be of great benefit in a multi-investigator trial. The study of large numbers of patients receiving amiodarone is probably necessary to observe the more rare adverse effects such as pulmonary toxicity in sufficient numbers. Sampling of serum drug concentrations should be more frequent during the first month of follow-up. The assumption that steady state will not be reached in the first year of therapy should be applied to the analysis of pharmacokinetic and dynamic data in future studies. Where practical studies should be extended to least at two years. The preliminary observation that alterations occur in lipid and carbohydrate metabolism, should be confirmed and the distribution of cholesterol in serum lipoproteins during amiodarone therapy determined. Double baseline measurements of fasting serum cholesterol, triglycerides and glucose concentrations would aid in assessing how much of the variability in these parameters which could not be ascribed to amiodarone. Although the change observed in serum creatinine was not clinically relevant during the first year, it would be of interest to observe if further deterioration takes place. The use of sophisticated techniques to monitor renal blood flow and ejection fraction in patients would be expensive but might provide information about the effect of amiodarone on renal function.

The possible mechanisms by which amiodarone could affect SOD activity should be taken into account when designing future studies. The drug could be directly inhibiting the enzyme. In this case endogenously produced free radicals from the oxidative metabolism of xenobiotics or activated oxygen

species extruded from mitochondrial metabolism could be responsible for cellular damage. The higher baseline SOD activity in patients who developed pulmonary activity might be indicative of a higher metabolic production of such free radicals in these patients. A fall in SOD activity could result from either an inhibition of the enzyme by drug or metabolite or an overwhelming of its capacity by a surplus of free radicals produced during hepatic drug metabolism. The possibility exists that like paraquat, amiodarone may induce the production of SOD in some individuals depending on their metabolic pathways. The contrary could occur in patients producing enough toxic metabolite to overwhelm SOD activity, as occurs with toxic doses of paraquat (Montgomery 1977). Such possibilities make consideration of genetic polymorphisms of metabolism an important facet in future studies.

Genetic polymorphism of drug metabolism is a rapidly expanding field of study (Jacqz et al 1986). Often adverse effects previously labelled as hypersensitivity reactions have been shown to result from marked differences between the metabolic pathways of the patient and the population norm. Since the first report of genetic polymorphism in the oxidation of debrisoquine by Mahgoub et al (1977), the concept that different populations of fast and slow metabolisers exist has developed into an important part of the current understanding of drug toxicity. Test substances such as sparteine or caffeine are used to measure the pattern of oxidation in patients. This information helps to predict variations in the ability of a population of patients to metabolize certain classes of molecules and thereby aids in the safer use of many drugs. For example, phenytoin is not toxic itself, but is metabolized to a reactive arene oxide intermediate leading to hepatotoxicity in those patients with a genetic inability to clear this metabolite rapidly enough (Spielberg et al 1981). The inherited deficiency of enzymes needed to detoxify the oxidative metabolites of

some drugs such as sulfonamides, can now be diagnosed in relatives of patients with "idiosyncratic" reactions using lymphocyte cell cultures (Shear et al 1986). When unusual or "idiosyncratic" drug toxicity is evaluated, the fact that all patients are not necessarily part of a homogeneous population and may have significant variation in their oxidative metabolisms should be considered (Kalow 1987). In the case of amiodarone toxicity, di-desethylamiodarone (DDEA) might be considered as a possible toxic agent. It does not accumulate in human serum but knowledge is lacking of its concentrations in tissues. If this proved to be a toxic metabolite, metabolic polymorphism in its formation and degradation or excretion would have an important role in the development of toxicity.

The results of future clinical studies of SOD activity would be easier to assess if the variability of the SOD assay could be reduced using modern laboratory equipment with automated sampling techniques. Measurement of markers of lipid peroxidation such as 9,11-linoleic acid or malondialdehyde would provide corroborating evidence for or against a free radical mechanism in amiodarone toxicity (Crump et al 1985). Access to lung and liver tissue specimens for analysis of SOD activity would allow the comparison of activity in tissues exposed to amiodarone with that of erythrocytes. It would be of great interest to look for a polymodal distribution of SOD activity in a population not exposed to amiodarone. Should this occur it would confirm the finding that there was a difference at baseline between the patients who developed pulmonary toxicity and those who did not. It would also be of use to gather data on the P-450 profiles of patients prior to exposure to amiodarone. Perhaps the simplest method would be to document oxidative phenotype for compounds such as caffeine and debrisoquine. If a specific P-450 profile were found to be associated with an elevated SOD activity prior to exposure to amiodarone this

could point to metabolic differences which may be causing increased free radical production.

Animal models would also be appropriate tools for investigating the possible role of free radicals. In considering an animal for the development of a model of pulmonary toxicity the following factors indicate that the guinea pig would be well suited for this purpose. The guinea pig lung is highly susceptible to free radical damage induced by paraquat, with an LD_{50} of 30 mg/kg compared to 150 mg/kg for rats (Clark et al 1966). The guinea pig is not susceptible to the respiratory viruses which often complicate the assessment of lung pathology in laboratory rats (Percy 1980). It is one of the few animals which does not form ascorbic acid endogenously and therefore can be maintained in a vitamin C depleted state possibly potentiating the effects of free radicals on the system (Percy 1980). The use of a guinea pig model of pulmonary toxicity would provide the opportunity to obtain tissues for the comparison of erythrocyte SOD activity with tissue SOD activity and to compare drug concentrations in tissue and blood. A positive control such as a group of animals administered paraquat would provide pathological specimens with alterations known to be of free radical origin for comparison with those from amiodarone treated animals. Manipulation of the model with scavenger depletion such as vitamin C or selenium deprivation offers the opportunity to further delineate the mechanism of action. The ability of pharmacologic scavengers to ameliorate the damage would also support a free radical mechanism of toxicity.

Another avenue of investigation would require the aid of laboratories with the expertise to perform cell culture and electron spin resonance studies. Such laboratories would be in the best position to study the effects of amiodarone on tissues at the molecular level. This type of study might provide the strongest evidence for or against a free radical mechanism of toxicity.

4.5 Summary

The major goals of this study were attained. Concentration-response relationships for many of the effects of amiodarone were demonstrated and serum drug concentrations were correlated with dosing. Preliminary observation of changes in superoxide dismutase activity produced results which bear further investigation. It would be reasonable to proceed with further research: 1) to confirm the utility of using clinical parameters for estimating serum drug concentrations and predicting the development of adverse effects and; 2) to look for evidence supporting or refuting the involvement of free radicals in the production of the adverse effects of amiodarone.

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APPENDIX 1.

Protocol for UWO human ethics review

THE UNIVERSITY OF WESTERN ONTARIO

HEALTH SCIENCES STANDING COMMITTEE ON HUMAN RESEARCH

Mail 12 copies to the Vice-President Health Sciences, Room H113, Health Sciences Centre, The University of Western Ontario, London.

A.D. Sharma	Asst. Prof. Medicine	Medicine
Name of Principal Investigator	Title	Department
Cardiac Investigation Unit, University Hospital		519-663-3522
Address Box 5339, Terminal A, London, Ontario N6A 5A5		Telephone Number

Project Title: Observation of the effects of Amiodarone on intracellular free radical scavenging activity in man

Names/Titles of All investigators; name of faculty advisor if a student.

A.D. Sharma, Asst. Prof. Med

S.G. Carruther, Assoc. Prof. Med.

R.F. Delmaestro, Asst. Prof. Sugery

List all procedures to be done for the purpose of this study which are not part of the ordinary care of the subject.

None

Previous history of this protocol:

New X Modification ____

Annual ____ Previous # ____

Previous expiry date ____

Does this submission differ in any way from the previously approved protocol?

Yes ____ No ____

Explain any differences in your protocol.

Radioisotopes:

Will any radioactive material be used? No

What isotopes? ____

If yes; please see Guidelines for Exposure

Drugs:

Name all drugs and dosages to be used. Any new drugs must have HPB approval; note the IND number.

Amiodarone-standard doses

Subjects: (Explain in detail in the protocol.)

No. of subjects 40

Sources Clinic

Compensation None

Will the study involve any:

Minors? Yes ____ No X

Incompetent subjects: Yes ____ No X

Pregnant subjects: Yes ____ No X

Support:

Is this research funded? ____

OR APPLIED FOR? C.H.F., M.R.C.

Agency ____ Number ____

Date proposal due in agency Dec 1, 1983

THIS IS A COVER PAGE ONLY. COMPLETE ALL 5 PAGES, AND INCLUDE A COPY OF ANY CONSENT FORMS.

Revised September, 1982

Duration of Project: 2 years

Date of anticipated completion of study involving human subjects July 1985

Note to Researchers: The Health Sciences Standing Committee on Human Research was established to ensure that research involving human subjects is reviewed in accordance with the recommendations of the Senate of The University of Western Ontario. The Committee does not attempt to assess the legal validity of the consent form or provide any other legal advice.

Insert any additional pages required as page 2a, 3a, etc. Be brief. (In most cases no more than three pages should be needed).

1. SUMMARY OF PROPOSED RESEARCH: (insert pages 2a, b, as required)

This form must be filled out completely so that the Committee can evaluate the ethical issues that may not be addressed in a grant application or study protocol. The grant application does not usually serve the purpose. Medical terminology should be kept to a minimum to enable clearer understanding by lay persons serving on the committee.

The summary shall include a short descriptive title; a summary of the study design, to include specific manipulations, drug names, doses and routes, including any form of radiation, and the nature of operations, tests, interviews, etc. Attach a copy of any questionnaire to be used. Address the scientific validity of the study, and the appropriateness of performing the study on human subjects. Include selected references where appropriate. If grant summary (~500 words) available, please include.

Hypothesis to be tested (~ 100 words):

We hypothesize that the side-effects observed in patients receiving the drug, Amiodarone, may be caused by highly reactive chemical substances called free radicals. Since cells are normally protected from free radical damage by a system of scavenging enzymes, we would expect to observe the activity of these enzymes to be altered in patients taking Amiodarone. This would depend on whether the drug increases free radical formation or interferes with the scavenging enzymes. Either change would show that Amiodarone is capable of inducing damaged mediated by free radicals.

Summary of Research:

See attached sheet

Statistical Analysis: Is the study a Pilot Study? _____
Or a randomized controlled study? _____
Other ? Specify self-paired

If a randomized controlled study - sample size: _____ a error: ___
b error: ___
effect studied : ___
size of effect : ___

Summary of Research

Amiodarone is a very effective antiarrhythmic drug, only recently introduced in North America. Like all drugs it does have side-effects. The most important side-effects observed during ten years of extensive use in Europe have been small deposits in the cornea that do not effect vision, and alterations in thyroid metabolism. However, in its limited use in North America, it has also been found to alter liver metabolism and pulmonary function. The reported incidence of side-effects has also increased since clinical use started in North America. This may be due to the fact that the drug is used as a last resort in very sick patients who have arrhythmias which are very difficult to treat and are given higher doses than in Europe where it is used as a routine antiarrhythmic.

Tissue has been examined from the eyes, nerves and skin of patients taking amiodarone and all have been found to contain deposits of lipofuscin. This is a by-product of free radical damage to cell fats. Normally, free radicals formed from day to day in the cells are quickly inactivated by a system of scavenging enzymes. We wish to look for evidence that amiodarone causes directly or indirectly, either an increased rate of free radical formation or an inhibition of the ability of the scavenger enzymes to inactivete the free radicals normally formed in the cell.

To study this question, we will observe closely patients who will be starting on amiodarone at the arrhythmia clinic, because of the seriousness of their arrhythmias. These patients are normally followed for changes in blood tests indicating liver and thyroid function, as well as clinical examinations for changes in the cornea and lung function. We will draw an extra tube of blood at each regular clinic visit which would not necessitate any additional pain or inconvenience for the patients who will be having blood drawn as part of routine follow-up of amiodarone therapy. The extra blood will be analyzed for the level of amiodarone and for the activity of the free radical scavenging enzyme system. Any observed changes will

be correlated with the onset and severity of side-effects should they develop in the patients. By performing this analysis we hope to be able to discern from the activity of the scavenging enzyme, the patients who are at risk for developing side-effects. Such a marker would make this very powerful drug even more useful by allowing the selection of patients who are at less risk of side-effects thereby making the drug available for use in less severely ill patients who might benefit from its convenient once daily dosing.

Because the test for scavenging enzyme is not currently in broad clinical use, it is difficult to estimate the standard deviation in the normal population, and therefore difficult to estimate the number of patients needed to prove statistical significance. To have an 80% chance (beta=0.20) of proving a difference as large as one standard deviation between treated and untreated patients at the 5% level of stastical significance (alpha=0.05), it would take 36 patients in a study with a completely randomized design. However, since we will be measuring levels in the same patient before and after treatement, we will be able to use the self-paired t-test. This is likely to be twice as effecient as the randomized design so that only about 16 to 20 patients will be required to prove a 5% level of stastical significance.

2. SAMPLE OF PERSONS TO BE STUDIED

Describe the subjects for investigation, method of gathering them, place where research will be carried on; indicate who will be contacting the subjects. (Insert page 3a as required).

As explained in the summary of the research, there is a group of patients being placed on the new drug Amiodarone, at the University Hospital arrhythmia clinic. They are selected because of the dangerous nature of their arrhythmia and its resistance to treatment with conventional therapy. Because of the newness of this drug, the patients are already closely followed with regular clinic visits, blood work and examination of the cornea and pulmonary function. We will recruit the next 20 patients from the clinic in whom it has been decided to start Amiodarone therapy. They will be asked to agree to allowing us to draw an extra 15 ml (½ oz) of blood during blood taking for other routine tests at each of 7 visits over one year. This blood would then be suitably preserved and examined later for Amiodarone level and activity of the red blood cell free radical scavenging system. These will be the only tests not currently being done as part of routine follow-up of patients taking Amiodarone.

The research will be conducted at the University Hospital out-patient arrhythmia clinic and will be subject to all the routine procedures for maintenance of proper health record confidentiality. Any data used outside the clinic will be coded to blind the investigator and to further preserve confidentiality. We the investigators will be personally contacting the patients as they are chosen for therapy with Amiodarone. We will be asking if they are willing to assist us in the important task of understanding the side-effects of this drug so that it can be used more confidently in the therapy of arrhythmias.

3. RISKS INVOLVED: (Insert page 4 a as required).

(a) Discuss the risks and benefits of the proposed research.

The proposed research presents no increased risk to the patient beyond that of the side-effects of Amiodarone, a drug which would be given to the patient anyway because it has been deemed clinically necessary to manage their refractory arrhythmias. In point of fact, there will be no change in the patient follow-up from those patients taking the drug who are not in the study. Therefore, there is no change in the risks or benefits between these two groups.

(b) Describe the discomfort or incapacity incident to the proposed study.

The patient will not be able to discern any change from the standard out-patient care delivered to patients taking Amiodarone, other than two extra visits to the clinic.

(c) Will additional hospitalization or outpatient visits be required?

There is no hospitalization included in the protocol. Two extra out-patient vists to facilitate closer monitoring during the early period on the drug are envisaged.

(d) Are the procedures in the study used customarily for diagnostic and/or therapeutic purposes?

All procedures are standard clinically indicated methods for the diagnosis and follow-up of the patients taking this drug. The only alteration from standard follow-up will be a small increase in the volume of routine blood drawn. There will be no increase in the frequency of blood drawing.

(e) Describe facilities available to protect the health and safety of the subjects, and procedures for preserving confidentiality.

Patients will have the full support of the arrhythmia clinc staff, cardiologists and the University Hospital Invensive Care Unit. Files will be maintained in the standard mannner for the out-patient clinic and data from patients used outside the clinic will be coded and blinded from the investigators.

(f) Are any standard therapies or diagnostic procedures to be withheld for the purpose of this study? If so, discuss the balance between risk and possible benefit.

No therapy will be withheld.

(g) Will management or treatment be prolonged or delayed?

All management and therapy will follow the standard course.

6. PLANS FOR INFORMING SUBJECTS AND OBTAINING ADEQUATE, APPROPRIATE, INFORMED CONSENT (Add pages 5 a, b, as required; a copy of the consent form must be attached)

(a) Describe the explanation to be given to subjects before they agree to become participants in the project, and, for surveys circulated by mail, attach a copy of the explanatory letter to the subjects. A letter of information may be appropriate in addition to a consent form for all projects.

The doctor in the arrhythmia clinic will discuss the treatment of the patient's arrhythmia with Amiodarone and describe its side-effects as well as its importance in treating the life threatening arrhythmias that the patient has. He will ask the patient to agree to allowing a small amount of extra blood to be drawn with other routine blood work at follow-up visits. A written explanation will be given to the patient to keep and consent will also be given to the patient to sign.

(b) Are the patients competent to consent? Yes X No ____
If not describe the alternate source of consent.

(c) To the best of your knowledge, are the subjects of this research proposal also the subject of any other research investigator? NO

If the answer to the above question is YES, briefly describe the other investigations.

(d) Instruction for preparation of consent form: See consent form.

A recommended alternative to a lengthy consent form is a letter of explanation which the patient can keep, and a brief consent form which indicates the patient has read the letter of explanation. The consent form and/or letter shall include: Name of subject; statement that the subject consents to participate in a "research project" (or "investigation" or "study"); a fair description of the procedure to be used (e.g. three intravenous injections), a statement to the effect that the risks are minimal, or that there may be side effects. Known side effects shall be detailed, and where they are unknown this shall be stated. Discomfort or inconvenience shall be described. If radiation is involved, please indicate the nature and number of times exposure to radiation will occur, and the equivalency in chest x-rays. (See Guidelines on Ionizing Radiation). An offer should be made to answer any inquiries, including name and telephone number of the person who will reply. An instruction shall be included, that the subject is free to withdraw at any time without jeopardy to future care. A statement, to the effect that anonymity will be protected, shall be included. NO exculpatory language limiting the subject's legal rights or releasing the researcher from liability for negligence, may be included! If a consent form is thought to be unnecessary or inadvisable, the reason should be stated.

APPENDIX 2.

Information sheet for the amiodarone toxicity study

GEORGE J. KLEIN, M.D., F.R.C.P.(C), F.A.C.C. ARJUN D. SHARMA, M.D., F.R.C.P.(C)
ARRHYTHMIA SERVICE • CARDIAC INVESTIGATION UNIT
UNIVERSITY HOSPITAL • London, Ontario N6A 5A5

(519)663-3264

We have chosen to treat the serious irregularity in your heart rhythm with a new drug called Amiodarone. This was chosen because other drugs have not been effective in controlling the irregularity of your heart beat. Amiodarone has been shown to be very useful in treating dangerous irregularities of heart rate in thousands of patients like yourself. However, like all drugs, it does cause side-effects in some people. In the case of this drug, the most important are the following.

1) small deposits forming in the cornea of the eye which do not affect vision.

2) changes in the metabolism of thyroid hormone and in liver metabolism.

3) changes in lung function.

In order to make sure you are not adversely affected by these possible side-effects, a standard set of blood tests, eye examinations and breathing tests will be done on a regular basis. As yet no one has found the cause of these side-effects. We have recently developed a new blood test which might provide the answer to what causes these side-effects. We would like your permission to draw an extra 15 ml (1/2 oz) of blood at the time of other routine blood tests each time you visit us over the next year. The blood will be examined to determine if this new test has the potential to prevent side-effects before they happen.

Your first six regular clinic visits will be scheduled at 1, 2, 3, 6, 9 and 12 months after starting this drug. If you feel this to be inconvenient, you are not obliged to participate. If you choose to participate you are free to withdraw at any time without jeopardizing your care at the clinic. Any information used in the investigation will be coded for complete confidentiality. If you have any questions at any time, please call one of us (Dr. Pollak or Dr. Sharma) at University Hospital 663-3522.

Sincerely yours,

TP/dv

APPENDIX 3.

Consent form for the amiodarone toxicity study

CONSENT FOR PARTICIPATION IN AMIODARONE TOXICITY INVESTIGATION

I have read the attached letter and understand the explanation of the investigation. I agree to participate by allowing extra blood to be drawn at my regular clinic visits while I am taking Amiodarone. I understand that I am free to withdraw from the study at any time without jeopardy to my continued care, and that my name will not be used in any publication of the results.

DATE

SIGNATURE

APPENDIX 4.

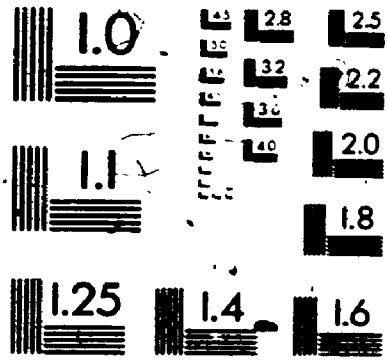
Example of clinical data collection sheet

Amiodarone Toxicity Study Dr. Pollak, Rm 6L21; Phone: 2999

Code No.___ Initials:_____ Chart No:_____ Arrhythmia:_____ Diagnoses:_____

Age:_____ Sex:_____ Weight:_____ Loading:_____

	Date							
Dose								
Other meds								
QTC								
HR								
Cornea								
Na								
K								
HCO3								
Creat								
Gluc								
Chol								
Trig								
Bili								
ALT								
AST								
Alk P								
FVC								
FEV1								
DCO								
PO2								
CO2								
T3RU								
T4RIA								
FTI								
T3RIA								
[Amio]								
[DEA]								
SOD								



APPENDIX 5.

Tabulated data for each parameter followed in the study

Dose of Amiodarone (mg) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	400	200	400	400	400	400	400
5	200	200	200	200	200	200	200
6	200	200	300	400	400	400	400
7	200	200	300	300	300	300	300
8	400	400	400	400	300	400	400
9	400	300	300	300	300	300	300
10	400	400	400	400	400	400	400
11	400	300	200	200	400	200	200
12	200	200	200	200	200	200	200
13	400	400	400	400	400	400	400
14	300	300	300	300	300	300	300
15	200	200	400	400	400	400	0
17	400	400	400	400	400	400	400
18	400	200	300	300	300	300	300
19	400	400	400	400	400	400	400
20	400	400	400	480	400	400	300
21	400	400	400	400	400	400	400
22	200	200	200	200	200	200	200
23	400	400	400	400	400	400	600
24	600	400	400	400	400	400	400
25	400	400	400	400	400	400	400
27	400	400	800	800	600	600	600
28	400	200	400	400	400	400	400
29	400	400	400	400	400	400	400
31	400	400	400	400	400	400	400
33	200	200	400	400	600	0	200
34	400	400	400	400	400	400	400
35	400	400	400	400	200	400	400
36	400	400	400	400	400	400	400
37	600	600	600	600	600	600	600
38	400	400	400	400	400	400	400
Mean	365	332	377	381	377	361	358
SD	105	105	115	114	102	115	126
CV	28.8	31.5	30.4	29.9	27.1	31.7	35.2
SEM	16.9	18.8	20.6	20.4	18.4	20.6	22.6

Percent Value of Parameter vs. Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	50	100	100	100	100	100
5	100	100	100	100	100	100	100
6	100	100	150	200	200	200	200
7	100	100	150	150	150	150	150
8	100	100	100	100	75	100	100
9	100	75	75	75	75	75	75
10	100	100	100	100	100	100	100
11	100	75	50	50	100	50	50
12	100	100	100	100	100	100	100
13	100	100	100	100	100	100	100
14	100	100	100	100	100	100	100
15	100	100	200	200	200	200	0
17	100	100	100	100	100	100	100
18	100	50	75	75	75	75	75
19	100	100	100	100	100	100	100
20	100	100	100	100	100	100	75
21	100	100	100	100	100	100	100
22	100	100	100	100	100	100	100
23	100	100	100	100	100	100	150
24	100	67	67	67	67	67	67
25	100	100	100	100	100	100	100
27	100	100	200	200	150	150	150
28	100	50	100	100	100	100	100
29	100	100	100	100	100	100	100
31	100	100	100	100	100	100	100
33	100	100	200	200	300	0	100
34	100	100	100	100	100	100	100
35	100	100	100	100	50	100	100
36	100	100	100	100	100	100	100
37	100	100	100	100	100	100	100
38	100	100	100	100	100	100	100
Mean	100	92	109	110	111	102	100
SD	0.0	16.4	35.4	36.4	47.2	36.4	33.2
CV	0.0	17.8	32.6	34.8	42.5	35.6	33.2

Amiodarone Concentration (mg/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0.000	2.028	2.311	2.594	3.230	2.683	1.704
5	0.000	2.821	2.952	3.727	5.244	4.585	2.274
6	0.000	0.576	0.741	0.516	0.571	0.693	0.656
7	0.000	1.631	1.547	2.146	2.517	1.350	1.611
8	0.000	1.007	1.039	1.219	1.977	1.618	2.202
9	0.000	2.035	1.758	1.344	1.475	1.188	1.783
10	1.020	1.373	4.490	3.175	1.083	1.156	2.281
11	0.000	0.586	0.986	0.685	0.648	0.859	0.836
12	0.000	1.520	1.644	1.210	2.801	2.316	2.886
13	0.000	1.820	2.147	2.857	4.966	4.837	5.131
14	0.000	1.113	1.710	2.822	2.304	3.144	2.644
15	0.000	1.276	4.473	5.877	2.652	2.385	0.524
17	0.000	0.491	0.501	0.590	0.608	0.675	0.728
18	0.000	3.002	3.054	3.126	4.107	3.884	2.928
19	0.000	2.091	3.359	3.225	5.079	5.731	4.452
20	0.000	1.907	2.190	2.286	3.045	2.771	2.741
21	0.000	2.760	2.311	2.286	3.172	2.574	4.314
22	0.000	1.749	1.465	1.171	1.311	1.472	1.423
23	0.000	1.586	1.695	2.046	2.419	1.611	1.452
24	0.000	2.109	1.494	2.152	1.417	2.857	2.827
25	0.000	1.061	1.497	1.933	3.054	3.589	4.133
27	0.000	1.403	4.759	3.369	3.879	4.691	4.739
28	0.000	0.304	0.721	0.530	0.773	0.985	1.583
29	0.000	1.183	1.792	1.625	1.408	1.527	1.842
31	0.000	1.184	1.211	1.446	1.412	1.733	1.504
33	0.000	0.973	1.834	2.968	3.932	0.747	1.425
34	0.000	2.782	3.083	3.591	3.726	3.243	3.156
35	0.000	1.195	1.803	1.388	1.072	1.200	1.636
36	0.000	1.741	2.033	2.326	3.709	2.963	5.155
37	0.000	1.991	2.081	2.195	2.369	2.663	3.038
38	0.000	5.007	3.277	2.256	2.246	3.568	4.393
Mean	0.033	1.667	2.128	2.216	2.523	2.429	2.516
SD	0.183	0.926	1.093	1.147	1.362	1.366	1.368
CV	0.0	54.9	51.4	51.8	54.0	56.2	54.0
SEM	0.033	0.166	0.196	0.206	0.245	0.245	0.244

Change in Parameter Since Last Visit

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0	2.028	0.283	0.283	0.636	-0.547	-0.979
5	0	2.821	0.130	0.775	1.517	-0.659	-2.311
6	0	0.576	0.165	-0.225	0.054	0.122	-0.037
7	0	1.631	-0.084	0.598	0.372	-1.167	0.261
8	0	1.007	0.033	0.179	0.758	-0.359	0.584
9	0	2.035	-0.276	-0.414	0.131	-0.287	0.595
10	0	0.353	3.117	-1.315	-2.092	0.073	1.125
11	0	0.586	0.399	-0.301	-0.037	0.211	-0.023
12	0	1.520	0.123	-0.434	1.591	-0.485	0.570
13	0	1.820	0.327	0.710	2.109	-0.129	0.294
14	0	1.113	0.596	1.112	-0.518	0.839	-0.500
15	0	1.276	3.196	1.404	-3.225	-0.267	-1.860
17	0	0.491	0.010	0.090	0.018	0.067	0.053
18	0	3.002	0.052	0.073	0.981	-0.223	-0.956
19	0	2.091	1.268	-0.134	1.854	0.652	-1.279
20	0	1.907	0.284	0.096	0.759	-0.274	-0.030
21	0	2.760	-0.449	-0.024	0.886	-0.598	1.740
22	0	1.749	-0.284	-0.294	0.139	0.161	-0.049
23	0	1.586	0.109	0.351	0.373	-0.808	-0.159
24	0	2.109	-0.615	0.658	-0.735	1.440	-0.030
25	0	1.061	0.436	0.436	1.121	0.535	0.543
27	0	1.403	3.356	-1.389	0.510	0.812	0.049
28	0	0.304	0.417	-0.191	0.243	0.212	0.598
29	0	1.183	0.609	-0.167	-0.217	0.119	0.316
31	0	1.183	0.028	0.235	-0.093	0.321	-0.229
33	0	0.973	0.861	1.134	0.964	-3.185	0.679
34	0	2.782	0.300	0.509	0.135	-0.483	-0.087
35	0	1.195	0.608	-0.415	-0.316	0.128	0.435
36	0	1.741	0.292	0.292	1.384	-0.746	2.191
37	0	1.991	0.090	0.114	0.174	0.294	0.375
38	0	5.006	-1.729	-1.022	-0.010	1.322	0.826
Mean	0	1.654	0.440	0.088	0.307	-0.094	0.087
SD							
CV							

DEA Concentration (mg/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0.000	1.186	1.175	1.163	2.078	1.814	1.522
5	0.000	1.098	1.336	1.510	2.063	1.830	1.536
6	0.000	0.498	0.597	0.537	0.605	0.580	0.557
7	0.000	0.803	0.869	1.115	1.577	0.902	1.199
8	0.000	0.517	0.482	0.466	1.072	1.181	1.292
9	0.000	0.930	0.991	0.759	0.896	0.815	1.251
10	0.395	1.105	1.293	1.234	0.681	0.556	1.106
11	0.000	0.593	0.830	0.677	0.697	0.982	0.826
12	0.000	0.415	0.486	0.563	0.951	0.753	0.562
13	0.000	0.346	0.468	0.564	0.912	0.785	1.480
14	0.000	0.747	0.815	1.744	1.577	1.518	2.108
15	0.000	0.748	1.762	2.421	1.634	1.773	0.827
17	0.000	0.388	0.376	0.461	0.381	0.376	0.436
18	0.000	0.791	1.078	1.107	1.168	1.478	1.484
19	0.000	0.600	1.202	1.184	1.640	1.599	1.711
20	0.000	0.521	0.650	0.781	1.142	1.307	1.506
21	0.000	0.670	0.890	1.139	1.852	1.684	2.692
22	0.000	0.696	0.634	0.640	0.634	0.716	0.906
23	0.000	1.062	1.198	1.340	1.530	1.461	1.401
24	0.000	0.804	0.639	1.297	0.424	1.326	1.528
25	0.000	0.613	0.846	1.078	1.541	1.979	2.172
27	0.000	1.040	2.063	2.117	2.609	2.857	3.062
28	0.000	0.229	0.380	0.458	0.490	0.670	1.091
29	0.000	0.671	0.959	0.995	1.083	1.389	1.271
31	0.000	0.485	0.469	0.558	0.750	0.913	1.001
33	0.000	0.559	0.954	1.479	1.317	0.865	1.033
34	0.000	1.070	1.111	1.215	1.567	1.424	1.739
35	0.000	0.623	0.776	0.777	0.655	0.781	0.900
36	0.000	0.809	0.968	1.128	1.460	1.384	2.038
37	0.000	0.856	0.842	1.187	1.561	2.002	2.313
38	0.000	2.330	1.743	1.374	1.555	2.090	2.381
Mean	0.013	0.768	0.932	1.067	1.229	1.283	1.446
SD	0.071	0.379	0.411	0.477	0.548	0.562	0.624
CV	0.0	49.4	44.1	44.7	44.6	43.8	43.2
SEM	0.013	0.068	0.074	0.086	0.098	0.101	0.112

Change in Parameter Since Last Visit

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0	1.186	-0.012	-0.012	0.915	-0.264	-0.292
5	0	1.098	0.238	0.174	0.553	-0.233	-0.294
6	0	0.498	0.099	-0.060	0.068	-0.025	-0.023
7	0	0.803	0.066	0.246	0.462	-0.675	0.297
8	0	0.517	-0.035	-0.016	0.606	0.109	0.111
9	0	0.930	0.061	-0.232	0.137	-0.081	0.436
10	0	0.710	0.188	-0.059	-0.553	-0.125	0.550
11	0	0.593	0.237	-0.153	0.020	0.286	-0.157
12	0	0.415	0.071	0.077	0.387	-0.198	-0.191
13	0	0.346	0.122	0.096	0.348	-0.127	0.695
14	0	0.747	0.068	0.929	-0.166	-0.060	0.591
15	0	0.748	1.015	0.658	-0.787	0.139	-0.946
17	0	0.388	-0.012	0.086	-0.080	-0.005	0.060
18	0	0.791	0.287	0.029	0.061	0.311	0.006
19	0	0.600	0.603	-0.018	0.456	-0.042	0.112
20	0	0.521	0.129	0.131	0.362	0.165	0.199
21	0	0.670	0.220	0.249	0.714	-0.168	1.008
22	0	0.696	-0.062	0.006	-0.006	0.082	0.191
23	0	1.062	0.137	0.142	0.190	-0.069	-0.060
24	0	0.804	-0.165	0.658	-0.873	0.902	0.202
25	0	0.613	0.232	0.232	0.463	0.438	0.194
27	0	1.040	1.023	0.054	0.492	0.248	0.105
28	0	0.229	0.150	0.078	0.032	0.180	0.421
29	0	0.671	0.288	0.036	0.088	0.306	-0.118
31	0	0.484	-0.015	0.089	0.192	0.163	0.088
33	0	0.559	0.395	0.525	-0.163	-0.452	0.169
34	0	1.070	0.042	0.103	0.353	-0.144	0.315
35	0	0.623	0.152	0.002	-0.123	0.127	0.119
36	0	0.809	0.160	0.160	-0.332	-0.076	0.654
37	0	0.856	-0.013	0.345	0.373	0.441	0.311
38	0	2.329	-0.587	-0.369	0.181	0.536	0.291
Mean	0	0.755	0.164	0.135	0.162	0.054	0.163
SD							
CV							

DEA/Amiodarone Ratio of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	-	0.585	0.224	0.448	0.643	0.676	0.893
5	-	0.389	0.453	0.405	0.393	0.399	0.676
6	-	0.865	0.806	1.040	1.060	0.837	0.849
7	-	0.492	0.562	0.520	0.626	0.668	0.744
8	-	0.514	0.464	0.382	0.542	0.730	0.587
9	-	0.457	0.564	0.565	0.608	0.686	0.702
10	-	0.805	0.288	0.389	0.629	0.481	0.485
11	-	1.009	0.842	0.987	1.074	1.143	0.988
12	-	0.273	0.296	0.466	0.339	0.325	0.195
13	-	0.190	0.218	0.197	0.184	0.162	0.288
14	-	0.671	0.477	0.618	0.685	0.483	0.798
15	-	0.586	0.394	0.412	0.616	0.743	1.576
17	-	0.790	0.750	0.782	0.626	0.557	0.599
18	-	0.263	0.353	0.354	0.284	0.381	0.507
19	-	0.287	0.358	0.367	0.323	0.279	0.384
20	-	0.293	0.297	0.341	0.375	0.472	0.549
21	-	0.243	0.385	0.498	0.584	0.695	0.624
22	-	0.398	0.433	0.547	0.484	0.486	0.637
23	-	0.669	0.707	0.655	0.632	0.907	0.964
24	-	0.381	0.426	0.603	0.299	0.464	0.541
25	-	0.578	0.379	0.558	0.504	0.551	0.526
27	-	0.742	0.434	0.628	0.673	0.609	0.625
28	-	0.754	0.527	0.864	0.634	0.680	0.689
29	-	0.667	0.535	0.613	0.769	0.910	0.890
31	-	0.409	0.387	0.386	0.531	0.527	0.666
33	-	0.574	0.520	0.498	0.335	1.158	0.725
34	-	0.384	0.360	0.338	0.421	0.439	0.551
35	-	0.522	0.430	0.560	0.611	0.651	0.550
36	-	0.465	0.234	0.485	0.394	0.467	0.395
37	-	0.430	0.405	0.541	0.659	0.752	0.761
38	-	0.465	0.532	0.609	0.692	0.586	0.542
Mean	-	0.517	0.453	0.537	0.556	0.608	0.655
SD	-	0.201	0.159	0.187	0.201	0.226	0.246
CV	-	38.8	35.1	34.7	36.2	37.1	37.6

Change in Parameter Since Last Visit

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	-	-	-0.361	0.225	0.195	0.033	0.217
5	-	-	0.063	-0.047	-0.012	0.006	0.276
6	-	-	-0.059	0.234	0.020	-0.223	0.012
7	-	-	0.069	-0.042	0.107	0.042	0.076
8	-	-	-0.050	-0.081	0.160	0.188	-0.143
9	-	-	0.107	0.001	0.043	0.079	0.015
10	-	-	-0.517	0.101	0.240	-0.148	0.004
11	-	-	-0.168	0.146	0.087	0.069	-0.155
12	-	-	0.023	0.170	-0.126	-0.014	-0.130
13	-	-	0.028	-0.021	-0.014	-0.021	0.126
14	-	-	-0.194	0.141	0.067	-0.202	0.315
15	-	-	-0.192	0.018	0.204	0.127	0.833
17	-	-	-0.040	0.031	-0.155	-0.069	0.042
18	-	-	0.089	0.001	-0.070	0.096	0.126
19	-	-	0.071	0.009	-0.044	-0.044	0.105
20	-	-	0.023	0.045	0.034	0.097	0.078
21	-	-	0.142	0.113	0.086	0.071	-0.030
22	-	-	0.035	0.114	-0.063	0.002	0.151
23	-	-	0.038	-0.052	-0.022	0.274	0.058
24	-	-	0.046	0.175	-0.303	0.165	0.076
25	-	-	-0.199	0.178	-0.053	0.047	-0.026
27	-	-	-0.308	0.195	0.044	-0.064	0.016
28	-	-	-0.227	0.337	-0.230	0.046	0.010
29	-	-	-0.032	0.077	0.157	0.141	-0.220
31	-	-	-0.022	-0.001	0.145	-0.005	0.139
33	-	-	-0.054	-0.022	-0.164	0.823	-0.433
34	-	-	-0.024	-0.022	0.082	0.018	0.112
35	-	-	-0.091	0.130	0.051	0.040	-0.100
36	-	-	-0.231	0.252	-0.091	0.073	-0.072
37	-	-	-0.025	0.136	0.118	0.093	0.010
38	-	-	0.066	0.077	0.083	-0.106	-0.044
Mean	-	-	-0.064	0.084	0.019	0.053	0.047
SD							
CV							

Heart Rate (bpm) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	55	64	64	80	64	60	61
5	65	64	64	66	62	63	71
6	143	80	97	75	77	77	70
7	53	76	56	84	71	51	59
8	50	59	49	51	53	55	55
9	75	76	74	62	69	71	76
10	55	64	83	70	67	66	67
11	64	54	45	68	45	47	41
12	60	64	65	62	60	61	63
13	73	58	64	70	50	50	50
14	76	70	71	75	72	67	66
15	68	57	59	106	109	107	100
17	99	64	64	69	75	67	65
18	85	70	69	68	67	63	60
19	65	61	59	57	56	60	56
20	120	80	76	72	67	64	61
21	99	59	56	70	58	69	83
22	67	66	64	64	63	65	61
23	80	71	70	62	64	60	56
24	102	80	80	72	75	61	67
25	103	72	72	72	72	80	82
27	100	68	80	83	57	60	62
28	123	46	45	44	46	46	45
29	95	65	60	67	55	55	55
31	99	50	53	56	64	58	61
33	122	61	59	88	66	68	54
34	80	70	64	60	60	61	52
35	150	70	95	87	69	64	58
36	70	55	54	53	53	52	43
37	90	79	72	66	63	63	67
38	79	69	63	61	53	62	50
Mean	86	66	66	68	64	63	62
SD	26.0	8.8	12.5	11.9	11.3	11.2	12.2
CV	30.3	13.4	18.9	17.4	17.8	17.8	19.7
SEM	4.7	1.6	2.2	2.1	2.0	2.0	2.2

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	116	116	145	116	109	111
5	100	98	98	102	95	97	109
6	100	56	68	52	54	54	49
7	100	143	106	158	134	96	111
8	100	118	98	102	106	110	110
9	100	101	99	83	92	95	101
10	100	116	151	127	122	120	122
11	100	84	70	106	70	73	64
12	100	107	108	103	100	102	105
13	100	79	88	96	68	68	68
14	100	92	93	99	95	88	87
15	100	84	87	156	154	157	147
17	100	65	65	70	76	68	66
18	100	82	81	80	79	74	71
19	100	94	91	88	86	92	86
20	100	67	63	60	56	53	51
21	100	60	57	71	59	70	84
22	100	99	96	96	94	97	91
23	100	89	88	78	80	75	70
24	100	78	78	71	74	60	66
25	100	70	70	70	70	78	80
27	100	68	80	83	57	60	62
28	100	37	37	36	37	37	37
29	100	68	63	71	58	58	58
31	100	51	54	57	65	59	62
33	100	50	48	72	54	54	44
34	100	88	80	75	75	76	65
35	100	47	63	45	46	43	39
36	100	79	77	76	76	74	61
37	100	88	80	73	70	70	74
38	100	87	80	77	67	78	63
Mean	100	83	82	86	80	79	78
SD	0.0	23.7	22.4	29.4	26.3	25.0	26.3
CV	0.0	28.7	27.4	34.0	32.8	31.7	33.8

Corrected QT Interval (msec) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	428	447	493	527	423	414	458
5	431	438	444	457	456	436	449
6	449	444	437	448	470	450	462
7	433	420	420	416	429	401	424
8	428	450	374	414	453	448	453
9	421	489	479	454	492	464	492
10	512	526	532	545	528	519	528
11	403	393	417	479	413	408	443
12	441	459	428	468	437	448	500
13	446	503	483	463	489	531	577
14	381	428	453	458	449	455	444
15	391	431	486	450	661	435	556
17	497	482	481	465	473	482	480
18	492	466	514	406	499	468	467
19	431	455	440	431	463	451	484
20	419	486	488	461	505	491	466
21	385	465	356	430	452	378	476
22	426	437	473	448	458	452	468
23	426	463	467	457	484	500	512
24	397	397	404	385	396	397	400
25	421	404	433	482	515	431	548
27	525	451	459	439	416	524	573
28	459	390	383	375	400	415	417
29	415	381	508	434	425	459	439
31	471	456	455	453	464	459	493
33	426	430	405	442	436	434	477
34	473	430	475	541	415	394	546
35	503	405	465	468	481	435	447
36	462	478	482	486	469	468	461
37	459	447	448	458	452	446	467
38	426	490	434	472	394	552	517
Mean	441	446	452	455	461	454	481
SD	36.9	35.0	41.1	37.5	51.0	42.1	45.0
CV	8.4	7.8	9.1	8.3	11.1	9.3	9.3
SEM	6.62	6.28	7.38	6.74	9.16	7.57	8.08

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	104	115	123	99	97	107
5	100	102	103	106	106	101	104
6	100	99	97	100	105	100	103
7	100	97	97	96	99	93	98
8	100	105	87	97	106	105	106
9	100	116	114	108	117	110	117
10	100	103	104	106	103	101	103
11	100	98	103	119	102	101	110
12	100	104	97	106	99	102	113
13	100	113	108	104	110	119	129
14	100	112	119	120	118	119	117
15	100	110	124	115	169	111	142
17	100	97	97	94	95	97	97
18	100	95	104	83	101	95	95
19	100	106	102	100	107	105	112
20	100	116	116	110	121	117	111
21	100	121	92	112	117	98	124
22	100	103	111	106	108	106	110
23	100	108	109	107	113	117	120
24	100	100	102	97	100	100	101
25	100	96	103	110	122	102	130
27	100	86	87	84	79	100	109
28	100	85	83	82	87	90	91
29	100	92	122	105	102	111	106
31	100	97	97	96	99	97	105
33	100	101	95	104	102	102	112
34	100	91	100	114	88	83	115
35	100	81	92	93	96	86	89
36	100	103	104	105	102	106	100
37	100	97	98	100	98	97	102
38	100	115	102	111	92	130	121
Mean	100	102	103	104	105	103	110
SD	0.0	9.6	10.0	10.3	15.4	10.0	11.8
CV	0.0	9.4	9.8	9.9	14.6	9.7	10.8

Microdeposit Grade (Mean of 2 eyes) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0	0	0	2	2	3	3
5	0	0	0	0.5	1	1	1
6	0	0	0	1	1.5	1	1
7	0	0	0	2	2	1.5	3
8	0	0	0	2	3	3	3
9	0	0	0.75	1	1	1.5	1.5
10	0	0	0	0.5	0.75	0	0
11	0	0	1	0.75	2.5	2	2.5
12	0	0.75	2	2	3	3	3
13	0	0	0	0	0.25	1	1
14	0	0	0.5	2	3	3	3
15	0	0	0.25	1	2	2	1.5
17	0	0.5	0.5	1	1	1.5	1
18	0	0	2	2	2	2	1
19	0	0	0.5	1	2	3	3
20	0	0	0	0	1	2.5	3.5
21	0	0	0	1	2.5	1.5	2.5
22	0	0	0	0	0.75	1	0.75
23	0	0	0	0.5	0.75	2	2
24	0	0	0	0.5	2.5	2	3
25	0	0	0	1.5	1.5	2.5	3
27	0	0	1	1	2	2.5	3
28	0	0.5	0	1	1	2	2.5
29	0	0	0	0	0.75	0.75	1.5
31	0	0	1	1.5	2	2	2.5
33	0	0	0	0.5	2	3	2
34	0	0	1	1	2	2	2
35	0	0.75	1	1.5	1	1	2
36	0	0	0	0.25	0.75	2	2.5
37	0	0	0	0.5	2.5	1.5	2.5
38	0	0	0	0	1.5	2	2
Mean	0.00	0.08	0.37	0.95	1.66	1.90	2.12
SD	0.00	0.22	0.58	0.68	0.78	0.78	0.89
CV	0.0	270.1	156.5	71.3	46.7	41.1	41.8
SEM	0.00	0.04	0.10	0.12	0.14	0.14	0.16

Change in Grade Since Last Visit

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0	0	0	2	0	1	0
5	0	0	0	1	1	0	0
6	0	0	0	1	1	-1	0
7	0	0	0	2	0	-1	2
8	0	0	0	2	1	0	0
9	0	0	0	0	0	1	0
10	0	0	0	1	0	-1	0
11	0	0	1	0	2	-1	1
12	0	1	1	0	1	0	0
13	0	0	0	0	0	1	0
14	0	0	1	2	1	0	0
15	0	0	0	1	1	0	-1
17	0	1	0	1	0	1	-1
18	0	0	2	0	0	0	-1
19	0	0	1	1	1	1	0
20	0	0	0	0	1	2	1
21	0	0	0	1	2	-1	1
22	0	0	0	0	1	0	0
23	0	0	0	1	0	1	0
24	0	0	0	1	2	-1	1
25	0	0	0	2	0	1	1
27	0	0	1	0	1	1	1
28	0	1	-1	1	0	1	1
29	0	0	0	0	1	0	1
31	0	0	1	1	1	0	1
33	0	0	0	1	2	1	-1
34	0	0	1	0	1	0	0
35	0	1	0	1	-1	0	-1
36	0	0	0	0	1	1	1
37	0	0	0	1	2	-1	1
38	0	0	0	0	2	1	0
Mean	0.00	0.08	0.29	0.58	0.71	0.23	0.23
SD							
CV							

DCO (mL/min/mmHg) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	ND						
5	20.5	21.7	20.9	20.5	20.6	17.3	24.8
6	29.4	28.6	27.9	27.3	26.5	25.6	34.1
7	22.5	20.6	22.9	24.4	18.6	21.3	23.3
8	23.3	19.5	20.0	20.4	21.4	20.0	24.4
9	21.1	24.6	25.5	26.1	24.6	26.4	31.0
10	21.0	18.6	26.3	26.1	21.1	27.2	26.2
11	27.6	24.2	29.2	24.5	20.1	21.5	32.1
12	19.8	20.0	22.2	21.4	16.6	24.1	23.3
13	25.3	24.5	23.7	19.9	15.1	13.8	19.4
14	17.9	18.2	18.5	19.1	16.9	21.5	22.9
15	21.1	20.8	20.5	14.7	19.7	16.4	21.9
17	19.3	28.8	19.1	19.6	16.4	21.0	19.3
18	15.8	15.7	13.6	13.6	12.5	15.8	16.7
19	17.0	13.6	12.5	11.4	13.0	13.6	13.2
20	29.5	30.0	23.7	22.1	29.5	30.5	30.5
21	17.8	15.8	16.6	16.8	20.0	17.0	19.1
22	20.6	19.8	21.5	22.1	25.1	25.9	22.5
23	25.4	22.5	29.1	28.9	33.0	35.5	31.8
24	16.9	14.9	12.8	12.9	18.6	16.2	14.9
25	35.6	24.8	28.7	32.7	32.5	29.0	31.2
27	25.5	26.6	26.4	26.1	21.3	29.6	18.6
28	36.5	34.7	40.5	37.8	36.9	37.8	35.1
29	23.6	23.6	23.6	28.1	26.2	19.3	18.3
31	28.2	21.7	28.8	24.6	22.9	22.3	28.6
33	26.8	29.7	28.8	29.1	25.9	23.5	23.2
34	19.9	14.8	18.8	15.8	15.4	11.2	12.7
35	27.9	23.8	22.3	23.5	22.9	21.2	19.6
36	25.4	22.0	21.5	20.9	20.6	19.9	18.8
37	21.8	18.7	18.7	18.5	14.6	15.7	15.8
38	23.9	24.0	25.6	23.2	25.0	27.6	25.6
Mean	23.6	22.2	23.0	22.4	21.8	22.3	23.3
SD	5.1	5.0	5.8	5.9	6.0	6.4	6.3
CV	21.8	22.7	25.3	26.5	27.4	28.7	26.9
SEM	0.94	0.92	1.06	1.08	1.09	1.17	1.14

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
5	100	106	102	100	100	85	121
6	100	97	95	93	90	87	116
7	100	92	102	109	83	95	104
8	100	84	86	88	92	86	104
9	100	117	121	124	117	125	147
10	100	89	125	125	101	130	125
11	100	88	106	89	73	78	116
12	100	101	112	108	84	122	118
13	100	97	94	79	60	54	77
14	100	102	103	107	95	120	128
15	100	99	97	69	93	77	104
17	100	150	99	102	85	109	100
18	100	99	86	86	79	100	106
19	100	80	73	67	77	80	78
20	100	101	80	75	100	103	103
21	100	89	93	94	112	96	107
22	100	96	105	107	122	126	110
23	100	89	115	114	130	140	125
24	100	88	76	76	110	96	88
25	100	70	81	92	91	82	88
27	100	104	103	102	83	116	73
28	100	95	111	103	101	103	96
29	100	100	100	119	111	82	78
31	100	77	102	87	81	79	101
33	100	111	107	109	97	88	87
34	100	74	94	79	77	56	64
35	100	85	80	84	82	76	70
36	100	87	84	82	81	78	74
37	100	86	86	85	67	72	73
38	100	101	107	97	105	116	107
Mean	100	95	98	95	93	95	100
SD	0.0	14.8	13.2	15.6	16.4	21.8	20.5
CV	0.0	15.6	13.5	16.4	17.7	22.9	20.6

FEV1 (L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	ND						
5	2.34	2.82	2.58	2.84	2.82	2.56	2.58
6	3.16	3.59	4.02	4.17	4.26	4.16	4.17
7	2.53	2.49	2.48	2.75	2.85	2.62	2.82
8	2.58	2.84	2.67	2.54	2.81	2.59	2.66
9	3.09	3.11	3.22	3.15	2.82	3.33	3.15
10	2.91	3.07	3.27	3.36	3.57	3.85	3.25
11	3.32	3.51	2.94	3.30	3.00	3.28	3.61
12	2.59	3.25	3.11	2.92	2.81	3.29	3.32
13	2.66	2.68	2.54	3.23	2.24	2.33	2.47
14	1.63	1.93	1.75	1.76	1.66	1.69	1.74
15	0.91	0.90	1.01	0.84	0.82	0.45	0.82
17	2.02	2.02	1.92	2.17	2.14	2.36	2.22
18	1.46	1.76	1.76	1.68	1.94	2.00	2.04
19	3.03	2.69	2.69	2.61	2.96	3.08	2.92
20	3.73	3.77	3.84	3.21	3.62	3.72	3.98
21	2.73	2.64	2.74	2.69	3.01	2.73	2.64
22	2.27	2.13	2.17	2.14	2.40	2.24	2.72
23	4.61	4.48	4.37	4.61	4.15	4.81	4.82
24	3.15	2.12	2.40	2.71	3.03	3.12	2.71
25	3.90	4.16	4.31	4.46	4.21	4.21	3.54
27	2.86	2.94	2.86	3.19	2.38	2.39	2.46
28	5.30	5.19	5.43	5.58	5.59	5.47	5.55
29	2.20	2.82	3.08	2.82	2.66	2.50	2.48
31	2.38	2.67	1.75	2.58	2.21	2.34	2.26
33	3.31	3.10	2.71	3.38	2.43	2.95	3.02
34	2.99	2.41	2.89	2.77	2.65	2.41	2.49
35	2.98	2.70	2.99	2.55	2.61	2.70	2.78
36	3.49	3.39	3.34	3.29	3.64	3.20	3.63
37	2.96	2.80	2.72	2.88	2.61	2.75	2.87
38	3.26	3.07	3.33	3.53	3.37	3.66	3.49
Mean	2.88	2.90	2.90	2.98	2.91	2.96	2.97
SD	0.86	0.84	0.90	0.91	0.90	0.96	0.91
CV	30.1	28.9	30.9	30.7	30.9	32.6	30.5
SEM	0.16	0.15	0.16	0.17	0.16	0.18	0.17

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4							
5	100	121	110	113	121	109	110
6	100	114	127	132	135	132	132
7	100	98	98	109	113	104	111
8	100	110	103	98	109	100	103
9	100	101	104	102	91	108	102
10	100	105	112	115	123	132	112
11	100	106	89	99	90	99	109
12	100	125	120	113	108	127	128
13	100	101	95	121	84	88	93
14	100	118	107	108	102	104	107
15	100	99	111	92	90	49	90
17	100	100	95	107	106	117	110
18	100	121	121	115	133	137	140
19	100	89	89	86	98	102	96
20	100	101	103	86	97	100	107
21	100	97	100	99	110	100	97
22	100	94	96	94	106	99	120
23	100	97	95	100	90	104	105
24	100	67	76	86	96	99	86
25	100	107	111	114	108	108	91
27	100	103	100	112	83	84	86
28	100	98	102	105	105	103	105
29	100	128	140	128	121	114	113
31	100	112	74	108	93	98	95
33	100	94	82	102	73	89	91
34	100	81	97	93	89	81	83
35	100	91	100	86	88	90	93
36	100	97	96	94	104	92	104
37	100	95	92	96	88	93	97
38	100	94	102	108	103	112	107
Mean	100	102	102	104	102	102	104
SD	0.0	13.0	14.1	12.1	14.7	17.2	13.5
CV	0.0	12.7	13.8	11.7	14.4	16.8	13.0

Forced Vital Capacity (L) Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
	ND						
5	3.46	3.99	3.89	3.87	4.06	3.91	3.59
6	3.94	4.30	4.70	5.01	5.00	4.89	4.99
7	3.81	3.77	3.65	3.92	4.05	4.02	4.18
8	3.02	3.20	3.06	3.07	3.32	3.18	3.10
9	4.23	4.19	4.42	4.47	3.74	4.44	4.23
10	3.83	4.40	4.37	4.28	4.84	5.13	4.51
11	5.14	5.12	4.69	4.88	4.49	5.17	5.49
12	3.38	4.29	4.07	3.81	3.54	4.40	4.41
13	3.36	3.32	3.26	2.58	2.85	2.87	3.01
14	2.26	2.62	2.56	2.33	2.36	2.21	2.62
15	1.64	1.57	1.64	1.53	1.34	1.47	1.60
17	3.13	3.26	3.08	3.39	3.20	3.56	3.39
18	2.78	2.95	2.56	2.61	2.95	3.25	3.74
19	4.22	4.29	3.98	3.82	3.98	4.28	4.17
20	5.36	5.48	5.09	4.66	5.09	5.10	5.52
21	3.56	3.20	3.43	3.34	3.78	3.36	3.40
22	2.82	2.67	2.77	2.77	3.10	2.94	3.05
23	4.96	4.92	4.97	5.31	5.08	5.40	5.63
24	4.03	2.96	3.08	3.63	4.05	4.13	3.71
25	4.63	5.14	5.30	5.46	5.31	5.16	4.29
27	3.52	3.58	3.60	4.10	3.21	3.13	3.12
28	6.68	6.43	6.84	6.92	6.80	6.67	6.65
29	2.87	3.04	3.83	3.72	3.46	3.20	3.18
31	2.82	3.00	2.53	3.10	2.85	2.88	2.78
33	4.31	3.90	3.80	4.40	3.85	3.85	3.96
34	4.14	3.52	4.34	4.10	3.98	3.58	3.83
35	3.67	3.53	3.68	3.31	3.29	3.50	3.71
36	5.42	5.20	5.14	5.08	4.97	4.97	5.23
37	3.79	3.82	3.70	3.72	3.58	3.73	3.84
38	4.66	4.64	4.93	4.89	4.83	4.92	5.03
Mean	3.85	3.88	3.90	3.94	3.90	3.98	4.00
SD	1.04	1.02	1.06	1.09	1.06	1.09	1.06
CV	27.0	26.3	27.2	27.8	27.2	27.4	26.6
SEM	0.19	0.19	0.19	0.20	0.19	0.20	0.19

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
5	100	115	112	112	117	113	104
6	100	109	119	127	127	124	127
7	100	99	96	103	106	106	110
8	100	106	101	102	110	105	103
9	100	99	104	106	88	105	100
10	100	115	114	112	126	134	118
11	100	100	91	95	87	101	107
12	100	127	120	113	105	130	130
13	100	99	97	77	85	85	90
14	100	116	113	103	104	98	116
15	100	96	100	93	82	90	98
17	100	104	98	108	102	114	108
18	100	106	92	94	106	117	135
19	100	102	94	91	94	101	99
20	100	102	95	87	95	95	103
21	100	90	96	94	106	94	96
22	100	95	98	98	110	104	108
23	100	99	100	107	102	109	114
24	100	73	76	90	100	102	92
25	100	111	114	118	115	111	93
27	100	102	102	116	91	89	89
28	100	96	102	104	102	100	100
29	100	106	133	130	121	111	111
31	100	106	90	110	101	102	99
33	100	90	88	102	89	89	92
34	100	85	105	99	96	86	93
35	100	96	100	90	90	95	101
36	100	96	95	94	92	92	96
37	100	101	98	98	94	98	101
38	100	100	106	105	104	106	108
Mean	100	101	102	103	102	104	105
SD	0.0	10.1	11.3	11.7	11.7	12.1	11.7
CV	0.0	10.0	11.1	11.4	11.5	11.7	11.2

PO2 (Torr) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	76	75	75	74	74	74	74
5	78	79	80	73	89	87	84
6	88	85	81	77	90	83	94
7	75	75	80	81	81	90	88
8	75	74	79	76	86	84	84
9	71	75	76	72	76	77	83
10	82	83	84	82	73	85	72
11	78	83	72	72	80	75	76
12	76	74	69	71	77	72	77
13	84	74	73	66	73	77	75
14	76	83	81	78	81	75	81
15	69	63	67	48	51	50	55
17	81	63	73	73	75	63	69
18	75	78	66	76	67	89	78
19	79	83	79	79	71	70	77
20	95	92	92	79	81	81	77
21	81	75	77	82	74	72	86
22	58	72	81	76	89	80	66
23	86	97	91	89	90	99	94
24	74	67	74	67	83	77	76
25	75	64	72	83	85	75	79
27	77	84	78	75	80	82	81
28	84	79	81	87	84	95	76
29	85	87	92	83	73	87	86
31	77	69	65	69	88	79	77
33	92	82	80	81	79	72	72
34	80	69	76	69	74	68	74
35	72	75	87	84	73	78	83
36	94	77	74	71	89	90	85
37	86	72	84	76	70	75	70
38	82	90	74	66	81	77	78
Mean	79	77	78	75	79	79	78
SD	7.6	8.3	7.0	7.9	8.3	9.6	7.9
CV	9.5	10.7	9.0	10.4	10.6	12.2	10.1
SEM	1.4	1.5	1.3	1.4	1.5	1.7	1.4

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	99	99	97	97	97	97
5	100	101	103	94	114	112	108
6	100	97	92	88	102	94	107
7	100	100	107	108	108	120	117
8	100	99	105	101	115	112	112
9	100	106	107	101	107	108	117
10	100	101	102	100	89	104	88
11	100	106	92	92	103	96	97
12	100	97	91	93	101	95	101
13	100	88	87	79	87	92	89
14	100	109	107	103	107	99	107
15	100	91	97	70	74	72	80
17	100	78	90	90	93	78	85
18	100	104	88	101	89	119	104
19	100	105	100	100	90	89	97
20	100	97	97	83	85	85	81
21	100	93	95	101	91	89	106
22	100	124	140	131	153	138	114
23	100	113	106	103	105	115	109
24	100	91	100	91	112	104	103
25	100	85	96	111	113	100	105
27	100	109	101	97	104	106	105
28	100	94	96	104	100	113	90
29	100	102	108	98	86	102	101
31	100	90	84	90	114	103	100
33	100	89	87	88	86	78	78
34	100	86	95	86	93	85	93
35	100	104	121	117	101	108	115
36	100	82	79	76	95	96	90
37	100	84	98	88	81	87	81
38	100	110	90	80	99	94	95
Mean	100	98	99	96	100	100	99
SD	0.0	10.3	11.4	12.3	14.6	14.0	11.3
CV	0.0	10.5	11.6	12.9	14.6	14.0	11.4

PCO2 (Torr) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	33	35	35	34	33	33	34
5	32	35	38	34	39	34	36
6	32	35	37	36	37	38	37
7	37	38	36	32	35	34	38
8	37	37	37	37	35	37	35
9	41	41	39	42	43	40	42
10	36	35	36	32	35	34	38
11	37	35	39	39	38	42	40
12	34	42	40	40	41	39	39
13	31	36	37	37	36	34	32
14	35	37	37	37	37	40	41
15	35	31	35	37	33	35	37
17	37	39	42	41	41	39	38
18	35	36	38	38	40	37	42
19	33	37	36	37	35	31	35
20	35	37	37	39	38	38	39
21	32	33	33	34	34	33	35
22	39	37	40	38	37	41	41
23	39	39	41	40	42	41	41
24	39	35	38	39	38	35	38
25	35	36	35	34	35	33	37
27	36	39	37	38	35	34	36
28	38	40	41	39	39	37	38
29	36	37	37	36	38	29	31
31	37	39	41	39	41	45	37
33	28	35	39	36	33	37	39
34	35	34	39	38	38	40	43
35	35	30	32	34	34	34	35
36	32	35	33	31	32	35	30
37	37	37	34	36	38	39	35
38	37	37	34	34	35	35	38
Mean	35	36	37	37	37	37	37
SD	2.8	2.6	2.6	2.7	2.9	3.5	3.2
CV	7.9	7.1	6.9	7.5	7.8	9.7	8.5
SEM	0.50	0.47	0.46	0.49	0.52	0.63	0.57

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	106	106	103	100	100	103
5	100	109	119	106	122	106	113
6	100	109	116	113	116	119	116
7	100	103	97	86	95	92	103
8	100	100	100	100	95	100	95
9	100	100	95	102	105	98	102
10	100	97	100	89	97	94	106
11	100	95	105	105	103	114	108
12	100	124	118	118	121	115	115
13	100	116	119	119	116	110	103
14	100	106	106	106	106	114	117
15	100	89	100	106	94	100	106
17	100	105	114	111	111	105	103
18	100	103	109	109	114	106	120
19	100	112	109	112	106	94	106
20	100	106	106	111	109	109	111
21	100	103	103	106	106	103	109
22	100	95	103	97	95	105	105
23	100	100	105	103	108	105	105
24	100	90	97	100	97	90	97
25	100	103	100	97	100	94	106
27	100	108	103	106	97	94	100
28	100	105	108	103	103	97	100
29	100	103	103	100	106	81	86
31	100	105	111	105	111	122	100
33	100	125	139	129	118	132	139
34	100	97	111	109	109	114	123
35	100	86	91	97	97	97	100
36	100	109	103	97	100	109	94
37	100	100	92	97	103	105	95
38	100	100	92	92	95	95	103
Mean	100	104	106	104	105	104	106
SD	0.0	8.7	9.8	8.7	8.2	10.6	10.1
CV	0.0	8.4	9.3	8.4	7.8	10.2	9.5

Serum ALT (U/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	25	66	62	58	101	93	77
5	33	28	23	37	30	25	48
6	8	11	10	10	13	11	10
7	42	30	34	38	25	41	57
8	24	22	28	28	27	35	36
9	13	11	13	11	15	13	13
10	16	18	25	23	21	20	20
11	17	18	26	22	20	26	20
12	16	22	22	20	26	24	16
13	21	25	34	38	44	41	43
14	23	25	27	30	70	23	24
15	15	20	27	39	136	43	27
17	35	23	22	20	25	18	20
18	25	27	29	21	74	43	45
19	20	27	55	34	58	66	69
20	44	35	43	59	62	64	91
21	59	84	89	61	137	124	132
22	21	27	28	33	30	27	31
23	17	12	14	16	18	20	25
24	21	34	49	25	31	35	31
25	28	29	34	38	42	38	31
27	39	22	23	38	137	97	114
28	52	67	89	116	300	231	716
29	33	23	23	382	56	195	59
31	37	23	16	30	30	34	54
33	19	20	29	39	41	32	47
34	16	39	17	18	30	30	34
35	22	14	19	24	22	18	15
36	19	27	79	130	105	102	116
37	40	22	31	18	20	33	39
38	21	136	66	46	42	33	46
Mean	26	32	35	37	58	48	68
SD	12.2	25.6	21.7	26.8	59.2	44.6	126.4
CV	46.4	79.7	61.4	71.8	102.6	92.9	185.2
SEM	2.2	4.7	4.0	4.9	10.8	8.1	23.1

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	264	248	232	404	372	308
5	100	85	70	112	91	76	145
6	100	138	125	125	163	138	125
7	100	71	81	90	60	98	136
8	100	92	117	115	113	146	150
9	100	85	100	85	115	100	100
10	100	113	156	144	131	125	125
11	100	106	153	129	118	153	118
12	100	138	138	125	163	150	100
13	100	119	162	181	210	195	205
14	100	109	117	130	304	100	104
15	100	133	180	260	907	287	180
17	100	66	63	57	71	51	57
18	100	108	116	84	296	172	180
19	100	135	275	170	290	330	345
20	100	80	98	134	141	145	207
21	100	142	151	103	232	210	224
22	100	129	133	157	143	129	148
23	100	71	82	94	106	118	147
24	100	162	233	119	148	167	148
25	100	104	120	136	150	136	111
27	100	56	59	97	351	249	292
28	100	129	171	223	577	444	1377
29							
31	100	62	43	81	81	92	146
33	100	105	153	205	216	168	247
34	100	244	106	113	188	188	213
35	100	64	86	109	100	82	68
36	100	142	413	684	553	537	611
37	100	55	78	45	50	83	98
38	100	648	314	219	200	157	219
Mean	100	132	145	152	222	180	221
SD	0.0	108.7	81.8	113.3	184.8	112.3	242.8
CV	0.0	82.5	56.5	74.6	83.1	62.5	109.8

* not analysed because of biliary obstruction

Serum AST (U/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	17	42	35	27	44	44	45
5	77	51	25	31	29	28	25
6	10	12	13	13	14	14	14
7	30	25	33	41	23	28	28
8	22	21	23	24	25	27	25
9	16	15	15	15	17	18	15
10	12	21	20	22	19	24	20
11	18	18	22	20	22	22	20
12	19	23	23	22	28	29	21
13	18	21	23	24	29	31	31
14	22	23	28	28	42	24	25
15	19	27	30	34	53	48	34
17	26	17	17	18	19	18	19
18	18	20	22	16	45	24	37
19	21	18	39	24	44	45	52
20	26	27	35	41	42	53	68
21	37	60	58	49	84	84	110
22	17	20	21	23	23	21	20
23	49	14	16	17	21	14	14
24	22	20	29	23	24	29	26
25	19	20	22	23	22	25	20
27	23	17	17	25	54	59	55
28	36	37	47	56	150	107	454
29	*23*	*24*	*25*	*398*	*37*	*171*	*29*
31	23	27	18	24	24	27	57
33	18	19	21	24	21	24	28
34	14	25	17	20	21	23	29
35	14	14	19	19	16	15	16
36	15	25	54	83	75	76	51
37	105	22	19	17	21	29	32
38	15	46	31	23	25	22	30
Mean	26	25	26	28	36	34	47
SD	19.8	11.3	11.2	14.4	27.5	21.9	79.4
CV	76.3	45.5	42.5	52.1	76.5	63.8	167.6
SEM	3.6	2.1	2.0	2.6	5.0	4.0	14.5

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	247	203	159	259	259	265
5	100	66	32	40	38	36	32
6	100	120	130	130	140	140	140
7	100	83	110	137	77	93	93
8	100	95	105	109	114	123	114
9	100	94	94	94	106	113	94
10	100	175	167	183	158	200	167
11	100	100	122	111	122	122	111
12	100	121	121	116	147	153	111
13	100	117	128	133	161	172	172
14	100	105	127	127	191	109	114
15	100	142	158	179	279	242	179
17	100	65	65	69	73	69	73
18	100	111	122	89	250	133	206
19	100	86	186	114	210	214	248
20	100	104	135	158	162	204	262
21	100	162	157	132	227	227	297
22	100	118	124	135	135	124	118
23	100	29	32	35	43	29	29
24	100	91	132	105	109	132	118
25	100	105	113	121	116	132	105
27	100	74	74	109	235	257	239
28	100	103	131	156	417	297	1261
29							
31	100	117	78	104	104	117	248
33	100	106	117	133	117	133	156
34	100	179	121	143	150	164	207
35	100	100	136	136	114	107	114
36	100	167	360	553	500	507	340
37	100	21	18	16	20	28	30
38	100	307	207	153	167	147	200
Mean	100	117	127	133	165	159	195
SD	0.0	57.1	62.9	88.5	103.1	94.0	216.7
CV	0.0	48.8	49.6	66.7	62.6	58.9	111.3

* not analysed because of biliary obstruction

Serum ALK (U/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	74	68	60	52	61	62	69
5	108	108	108	108	109	128	204
6	68	72	69	66	71	82	84
7	81	73	75	76	80	57	49
8	82	86	66	80	94	87	84
9	65	65	61	64	71	61	59
10	105	91	75	78	85	83	75
11	75	91	128	96	74	88	79
12	97	112	109	101	105	92	92
13	85	107	127	125	121	129	123
14	98	105	116	128	175	126	124
15	94	105	85	122	140	137	140
17	142	137	125	125	129	122	115
18	104	115	125	115	118	132	126
19	83	82	88	66	81	106	71
20	90	89	100	101	126	100	101
21	56	79	71	77	77	89	92
22	85	92	82	83	71	76	88
23	69	73	72	71	97	74	81
24	130	135	133	135	146	125	111
25	85	85	80	75	81	76	102
27	79	89	83	85	119	86	93
28	59	65	64	65	66	64	302
29	*377*	*207*	*114*	*258*	*278*	*432*	*268*
31	88	84	107	107	102	123	108
33	70	69	65	76	74	82	77
34	88	81	71	66	81	100	81
35	128	105	101	91	93	102	84
36	108	135	137	139	134	119	107
37	108	102	102	101	114	104	105
38	85	91	84	83	87	77	70
Mean	88	93	92	92	99	96	103
SD	21.5	20.3	24.2	24.1	27.8	23.8	47.6
CV	24.3	21.8	26.3	26.2	28.0	24.7	46.1
SEM	3.9	3.7	4.4	4.4	5.1	4.4	8.7

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	92	81	70	82	84	93
5	100	100	100	100	101	119	189
6	100	106	101	97	104	121	124
7	100	90	92	94	99	70	60
8	100	105	80	98	115	106	102
9	100	100	94	98	109	94	91
10	100	87	71	74	81	79	71
11	100	121	171	128	99	117	105
12	100	115	112	104	108	95	95
13	100	126	149	147	142	152	145
14	100	107	118	131	179	129	127
15	100	112	90	130	149	146	149
17	100	96	88	88	91	86	81
18	100	110	120	111	113	127	121
19	100	99	106	80	98	130	86
20	100	99	111	112	140	111	112
21	100	141	127	138	138	159	164
22	100	108	96	98	84	89	104
23	100	106	104	103	141	107	117
24	100	104	102	104	112	96	85
25	100	131	123	115	125	117	157
27	100	113	105	108	151	109	118
28	100	110	108	110	112	108	512
29							
31	100	95	122	122	116	140	123
33	100	99	93	109	106	117	110
34	100	92	81	75	92	114	92
35	100	82	79	71	73	80	66
36	100	125	127	129	124	110	99
37	100	94	94	94	106	96	97
38	100	140	129	128	134	118	108
Mean	100	107	106	105	114	111	123
SD	0.0	14.8	21.7	20.2	24.2	21.8	78.9
CV	0.0	13.8	20.5	19.2	21.3	19.7	63.9

* not analysed because of biliary obstruction

Total Serum Bilirubin (mmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	8.1	6.9	6.8	6.7	6.7	6.5	6.0
5	13.3	7.9	8.0	11.3	7.2	6.5	6.8
6	9.8	8.7	7.6	7.9	5.9	6.6	8.3
7	7.8	7.8	9.1	6.7	6.6	9.6	9.8
8	11.0	7.7	8.1	10.5	7.5	8.4	9.5
9	8.3	8.4	8.5	8.2	5.2	7.6	10.0
10	3.0	0.2	4.6	8.1	23.5	16.1	8.3
11	8.2	8.2	6.9	9.8	7.1	6.9	9.3
12	1.2	6.0	6.9	6.7	7.3	9.2	8.3
13	12.3	7.2	5.2	7.3	7.7	5.5	10.0
14	6.0	4.6	2.6	6.3	5.3	3.9	4.0
15	11.6	7.6	14.9	9.5	8.1	13.0	12.2
17	7.0	6.5	5.7	6.2	6.0	6.4	6.3
18	11.0	7.0	12.9	11.2	10.7	15.2	13.0
19	8.3	9.1	6.9	5.4	4.1	8.1	7.4
20	10.9	11.1	11.2	10.5	11.3	11.3	13.5
21	12.9	11.3	8.3	12.1	9.1	11.6	16.6
22	7.0	4.0	3.9	6.2	5.9	5.4	5.5
23	50.0	18.9	37.4	18.0	17.0	25.3	19.9
24	9.2	7.8	5.7	5.3	7.8	10.1	6.4
25	6.6	6.9	6.8	10.6	8.6	10.9	11.4
27	11.0	13.7	6.3	6.1	8.5	6.3	5.7
28	12.9	8.9	11.1	12.1	12.0	8.9	10.3
29	*14*	*17.4*	*12.1*	*17.2*	*25.7*	*67.3*	*19.4*
31	12.9	10.9	7.2	6.7	6.1	4.3	15.1
33	8.6	6.2	8.8	8.1	5.2	5.2	4.6
34	13.0	10.8	8.8	10.9	10.1	6.2	9.8
35	15.8	27.8	13.2	18.4	19.4	11.1	14.6
36	11.7	15.3	12.6	9.9	16.7	8.1	11.3
37	6.2	4.3	6.0	5.1	6.0	4.8	5.5
38	8.7	6.8	8.3	8.2	9.6	7.2	9.8
Mean	10.8	9.0	9.1	9.0	9.1	8.9	9.6
SD	8.1	5.0	6.1	3.3	4.5	4.4	3.8
CV	74.5	55.9	66.7	36.5	50.1	49.0	38.9

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	85	84	83	83	80	74
5	100	59	60	85	54	49	51
6	100	89	78	81	60	67	85
7	100	100	117	86	85	123	126
8	100	70	74	95	68	76	86
9	100	101	102	99	63	92	120
10	100	7	153	270	783	537	277
11	100	100	84	120	87	84	113
12	100	500	575	558	608	767	692
13	100	59	42	59	63	45	81
14	100	77	43	105	88	65	67
15	100	66	128	82	70	112	105
17	100	93	81	89	86	91	90
18	100	64	117	102	97	138	118
19	100	110	83	65	49	98	89
20	100	102	103	96	104	104	124
21	100	88	64	94	71	90	129
22	100	57	56	89	84	77	79
23	100	38	75	36	34	51	40
24	100	85	62	58	85	110	70
25	100	105	133	161	130	165	173
27	100	125	57	55	77	57	52
28	100	69	86	94	93	69	80
29							
31	100	84	56	52	47	33	117
33	100	72	102	94	60	60	53
34	100	83	68	84	78	48	75
35	100	176	84	116	123	70	92
36	100	131	108	85	143	69	97
37	100	69	97	82	97	77	89
38	100	78	95	94	110	83	113
Mean	100	98	102	109	123	120	119
SD	0.0	81.7	93.2	94.1	159.4	150.5	116.8
CV	0.0	83.4	91.2	86.4	129.9	125.8	98.5

Serum Cholesterol (mmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	ND						
5	ND						
6	ND						
7	ND						
8	ND						
9	6.3	5.8	5.3	5.1	4.5	6.1	4.4
10	4.1	5.8	5.8	5.5	5.4	5.7	5.4
11	4.8	4.9	4.2	4.4	5.0	4.2	4.5
12	4.8	5.0	5.4	4.7	5.8	5.7	5.2
13	5.2	4.6	5.1	5.0	5.6	5.1	5.0
14	6.7	7.2	5.4	7.4	7.9	7.7	7.7
15	5.9	5.3	5.7	6.9	4.3	5.2	4.4
17	5.6	5.5	5.1	5.4	4.8	5.8	4.9
18	4.7	5.3	5.9	5.1	6.0	6.3	6.1
19	4.6	4.6	5.8	5.5	6.1	6.0	5.6
20	3.7	4.9	5.9	5.7	5.0	5.0	4.8
21	3.6	4.4	4.4	3.8	3.5	4.5	4.6
22	6.9	7.6	7.7	7.3	7.2	7.5	7.6
23	4.4	4.8	4.5	4.2	4.3	4.7	4.8
24	4.8	4.8	5.1	6.1	4.9	6.6	6.0
25	4.5	5.6	5.7	5.8	6.2	5.8	5.6
27	5.4	5.7	6.4	6.3	5.8	5.8	5.9
28	2.4	1.9	2.3	2.1	2.4	2.4	2.5
29	4.5	4.4	6.0	5.8	4.6	4.0	4.8
31	2.9	5.6	5.1	5.2	5.4	5.4	5.4
33	7.8	6.9	7.8	7.8	7.4	5.9	6.6
34	5.2	5.2	5.6	6.4	5.9	6.0	6.2
35	5.8	5.6	7.0	5.9	6.9	6.6	6.6
36	4.8	5.6	4.6	3.8	5.2	5.3	4.2
37	4.2	6.3	6.9	6.1	6.4	6.1	6.2
38	7.4	9.2	8.5	8.2	8.3	8.2	8.1
Mean	5.0	5.5	5.7	5.6	5.6	5.6	5.5
SD	1.3	1.3	1.3	1.4	1.3	1.2	1.2
CV	25.5	23.9	22.3	24.3	23.8	21.3	22.2
SEM	0.25	0.26	0.25	0.27	0.26	0.24	0.24

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4							
5							
6							
7							
8							
9	100	92	84	81	71	81	70
10	100	141	141	134	132	139	132
11	100	102	88	92	104	88	94
12	100	104	113	98	121	119	108
13	100	88	98	96	108	98	96
14	100	107	81	110	118	115	115
15	100	90	97	117	73	88	75
17	100	98	91	96	86	104	88
18	100	113	126	109	128	134	130
19	100	100	126	120	133	130	122
20	100	132	159	154	136	135	130
21	100	122	122	106	97	125	128
22	100	110	112	106	104	109	110
23	100	109	102	95	98	107	109
24	100	100	106	127	102	138	125
25	100	124	127	129	138	129	124
27	100	106	119	117	107	107	109
28	100	79	96	88	100	100	104
29	100	98	133	129	102	89	107
31	100	193	176	179	186	186	186
33	100	88	100	100	95	76	85
34	100	100	108	123	113	115	119
35	100	97	121	102	119	114	114
36	100	117	96	75	108	110	88
37	100	150	164	145	152	145	148
38	100	124	115	111	112	111	109
Mean	100	111	115	113	113	115	112
SD	0.0	23.7	24.5	23.3	24.2	23.7	24.0
CV	0.0	21.4	21.2	20.6	21.4	20.6	21.3

Serum Triglycerides (mmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	ND						
5	ND						
6	ND						
7	ND						
8	ND						
9	1.55	1.85	2.15	1.51	1.63	1.16	2.32
10	1.92	8.20	9.00	5.10	1.79	2.44	6.60
11	1.64	1.34	2.28	1.36	1.00	1.90	1.24
12	0.66	1.40	1.30	1.15	2.06	1.54	1.36
13	1.00	0.73	1.41	1.92	2.10	1.60	1.79
14	2.02	1.40	1.30	1.14	1.33	1.94	2.04
15	1.20	2.48	1.24	3.23	2.08	2.52	1.26
17	2.46	2.22	2.05	2.33	2.22	1.20	1.84
18	0.89	0.98	1.08	0.79	1.92	1.45	2.26
19	1.18	1.07	3.40	1.15	2.05	1.86	1.42
20	2.43	2.63	3.46	2.30	1.83	1.47	2.05
21	1.97	3.76	3.87	1.95	3.35	2.03	3.33
22	1.70	2.73	2.28	1.43	1.64	2.60	1.96
23	1.24	0.88	1.07	1.06	1.69	0.60	0.57
24	0.52	0.61	0.61	0.89	0.64	0.34	0.89
25	1.72	1.02	1.80	2.65	4.56	2.95	2.64
27	1.91	1.68	3.88	1.24	2.06	2.48	2.01
28	0.37	0.62	0.97	0.38	0.34	0.45	1.10
29	1.70	1.26	2.15	0.98	1.08	0.45	0.77
31	1.27	1.94	1.48	1.27	2.03	1.80	1.80
33	2.24	3.15	3.10	4.10	3.03	1.99	1.91
34	1.00	1.97	2.06	2.92	2.27	1.92	2.39
35	2.02	1.55	2.48	1.59	1.50	3.97	2.66
36	3.70	3.54	2.80	2.14	2.84	2.28	2.88
37	0.77	1.00	1.15	0.96	0.74	1.07	0.94
38	2.22	7.32	4.28	3.28	3.08	4.80	6.58
Mean	1.59	2.21	2.41	1.88	1.96	1.88	2.19
SD	0.73	1.86	1.68	1.11	0.91	1.02	1.46
CV	46.1	84.2	69.9	59.3	46.4	54.5	67.0
SEM	0.14	0.36	0.33	0.22	0.18	0.20	0.29

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4							
5							
6							
7							
8							
9	100	119	139	97	105	75	150
10	100	427	469	266	93	127	344
11	100	82	139	83	61	116	76
12	100	212	197	174	312	233	206
13	100	73	141	192	210	160	179
14	100	69	64	56	66	96	101
15	100	207	103	269	173	210	113
17	100	90	83	95	90	49	79
18	100	110	121	89	216	163	254
19	100	91	288	97	174	158	120
20	100	108	142	95	75	60	84
21	100	191	196	99	170	103	169
22	100	161	134	84	96	153	115
23	100	71	86	85	136	48	46
24	100	117	117	171	123	65	171
25	100	59	105	154	265	172	153
27	100	88	203	65	108	130	105
28	100	168	262	103	92	422	297
29	100	74	126	58	64	26	45
31	100	153	117	100	160	142	142
33	100	141	140	183	135	89	85
34	100	197	206	292	227	192	239
35	100	77	123	79	74	197	132
36	100	96	76	58	77	62	78
37	100	130	149	125	96	139	122
38	100	330	193	148	139	216	296
Mean	100	140	158	128	136	127	150
SD	0.0	84.9	83.5	67.2	66.4	57.4	79.8
CV	0.0	60.7	52.7	52.7	48.8	45.2	53.2

Serum Glucose (mmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	6.3	9.7	8.6	8.1	9.9	9.6	10.0
5	8.6	7.4	8.3	5.1	5.8	7.5	6.7
6	5.5	5.2	5.5	5.1	5.4	5.3	4.5
7	6.8	5.4	4.9	6.2	6.1	5.2	5.8
8	5.6	5.3	5.2	5.9	5.0	5.4	6.3
9	4.9	4.7	4.6	5.1	5.4	5.3	5.3
10	4.7	5.7	7.0	5.3	5.0	4.9	5.1
11	5.1	4.1	4.3	5.2	4.8	4.5	5.1
12	4.8	5.9	6.0	6.6	5.3	5.8	5.9
13	7.8	5.2	5.8	5.3	6.8	5.8	5.1
14	5.1	4.9	4.8	5.5	4.8	5.1	5.1
15	6.0	7.9	3.6	13.5	15.3	13.1	8.6
17	5.1	4.5	4.9	5.0	5.2	5.3	5.8
18	5.5	5.6	5.1	4.0	5.2	4.4	5.5
19	7.3	5.1	6.7	6.3	7.2	7.3	4.9
20	6.3	5.1	4.0	4.1	5.3	4.3	4.3
21	5.6	5.7	6.7	5.9	5.4	6.1	6.9
22	6.7	6.6	4.2	4.8	5.2	5.1	6.3
23	5.7	5.8	4.8	4.5	4.9	4.4	4.5
24	10.2	9.6	11.2	6.6	6.9	6.5	4.3
25	5.9	5.0	4.9	4.8	4.8	5.1	6.1
27	5.5	4.9	6.1	5.3	6.7	4.7	4.8
28	5.3	5.2	5.1	5.0	5.5	5.2	5.0
29	4.7	5.0	5.5	6.2	5.9	5.9	4.7
31	14.9	12.7	8.3	7.2	7.3	8.5	10.9
33	5.5	5.7	4.9	6.0	5.2	5.2	6.6
34	4.6	19.8	21.5	19.8	16.4	10.9	9.6
35	7.1	5.1	5.6	5.4	4.9	4.4	5.6
36	5.7	5.3	4.9	4.7	5.2	4.8	5.5
37	6.6	5.1	4.9	5.1	5.1	5.1	5.5
38	5.8	6.6	4.3	6.8	5.8	5.6	5.5
Mean	6.3	6.4	6.1	6.3	6.4	6.0	6.0
SD	2.0	3.1	3.2	3.0	2.7	2.0	1.7
CV	32.0	47.5	52.8	48.2	43.0	33.7	27.6
SEM	0.36	0.55	0.58	0.54	0.49	0.36	0.30

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	154	137	129	157	152	159
5	100	86	73	59	67	87	78
6	100	95	100	93	98	96	82
7	100	79	72	91	90	76	85
8	100	95	93	105	89	96	113
9	100	96	94	104	110	108	108
10	100	121	149	113	106	104	109
11	100	80	84	102	94	88	100
12	100	123	125	138	110	110	123
13	100	67	74	68	87	74	65
14	100	96	94	108	94	100	100
15	100	132	60	225	255	218	143
17	100	88	96	98	102	104	114
18	100	102	93	73	95	80	100
19	100	70	92	86	99	100	67
20	100	81	63	65	84	68	68
21	100	102	120	105	96	109	123
22	100	99	63	72	78	78	94
23	100	102	84	79	86	77	79
24	100	94	110	65	68	64	42
25	100	85	83	81	81	86	103
27	100	89	111	96	122	85	87
28	100	98	96	94	104	98	94
29	100	106	117	132	126	126	100
31	100	85	56	48	49	57	73
33	100	104	89	109	95	95	120
34	100	430	467	430	357	237	209
35	100	72	79	76	69	62	79
36	100	93	86	82	91	84	96
37	100	77	74	77	77	77	83
38	100	118	77	121	104	100	98
Mean	100	107	104	107	108	100	100
SD	0.0	62.9	71.0	68.1	57.9	39.3	31.0
CV	0.0	58.7	68.6	63.5	53.8	39.3	31.1

Serum Creatine (mmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	93	115	102	95	102	104	105
5	113	126	141	142	126	134	143
6	71	72	73	78	72	68	68
7	88	118	100	102	111	107	105
8	78	89	81	94	100	91	92
9	110	108	111	114	108	108	109
10	138	122	123	125	122	124	116
11	89	90	108	97	109	98	101
12	100	116	120	110	119	114	101
13	87	107	118	103	120	114	123
14	116	115	119	101	112	107	97
15	149	165	169	173	153	155	138
17	99	99	115	99	101	101	97
18	91	105	111	107	115	113	142
19	141	128	134	161	142	138	129
20	87	122	99	123	96	104	104
21	136	158	143	147	154	148	147
22	89	84	80	83	82	78	84
23	95	104	115	124	119	108	125
24	91	113	131	128	121	135	144
25	107	101	103	105	110	112	113
27	115	118	124	127	148	142	143
28	131	112	116	117	108	114	100
29	76	89	133	177	163	164	155
31	97	94	78	79	86	77	88
33	115	98	102	114	99	108	99
34	107	103	105	107	121	109	124
35	101	97	108	105	111	108	139
36	116	119	115	110	118	112	105
37	124	132	140	139	175	165	138
38	93	86	96	123	101	139	110
Mean	105	110	113	114	117	116	116
SD	20.0	21.9	20.9	21.9	23.3	23.8	21.8
CV	19.1	19.9	18.5	19.2	19.9	20.5	18.9
SEM	3.58	3.94	3.76	3.94	4.19	4.27	3.92

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	124	110	102	110	112	113
5	100	112	125	126	112	119	127
6	100	101	103	110	101	96	96
7	100	134	114	116	126	122	119
8	100	114	104	121	128	117	118
9	100	98	101	104	98	98	99
10	100	88	89	91	88	90	84
11	100	101	121	109	122	110	113
12	100	116	120	110	119	114	101
13	100	123	136	118	138	131	141
14	100	99	103	87	97	92	84
15	100	124	113	116	103	104	93
17	100	100	116	100	102	102	98
18	100	115	122	118	126	124	156
19	100	91	95	114	101	98	91
20	100	140	114	141	110	120	120
21	100	116	105	108	113	109	108
22	100	94	90	93	92	88	94
23	100	109	121	131	125	114	132
24	100	124	144	141	133	148	158
25	100	94	96	98	104	105	106
27	100	103	108	110	129	123	124
28	100	85	89	89	82	87	76
29	100	117	175	154	214	216	204
31	100	97	80	81	89	79	91
33	100	85	89	99	86	94	86
34	100	96	98	100	113	102	116
35	100	96	107	104	110	107	138
36	100	103	99	95	102	97	91
37	100	106	113	112	141	133	111
38	100	92	103	132	109	149	116
Mean	100	106	110	111	114	113	113
SD	0.0	14.2	18.7	16.9	24.4	25.5	26.7
CV	0.0	13.4	17.0	15.3	21.4	22.6	23.6

Serum Sodium (meq/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	142	140	140	141	139	141	140
5	142	143	143	143	143	144	143
6	142	142	142	143	142	148	142
7	142	140	140	140	141	141	141
8	140	138	139	140	138	139	138
9	140	140	140	141	143	142	140
10	140	138	139	140	140	141	142
11	143	141	140	141	143	143	142
12	143	140	142	141	143	141	138
13	139	141	142	139	140	139	138
14	143	141	146	142	141	140	142
15	139	137	137	138	136	139	142
17	144	140	142	141	145	141	141
18	139	139	137	141	141	137	139
19	144	141	140	139	139	139	139
20	140	141	140	141	138	141	138
21	143	147	143	137	141	142	140
22	140	143	143	139	140	141	144
23	140	139	143	141	140	143	143
24	134	136	137	139	140	135	142
25	144	141	141	141	142	142	142
27	144	139	141	140	140	140	143
28	143	143	142	141	142	143	142
29	138	140	136	138	139	139	141
31	134	138	141	142	141	141	141
33	138	138	140	138	142	140	138
34	139	131	133	133	137	138	141
35	141	139	141	144	142	142	141
36	141	140	141	141	141	144	142
37	136	143	141	142	141	141	140
38	143	140	141	140	140	141	141
Mean	140.6	140.0	140.3	140.2	140.6	140.7	140.8
SD	2.7	2.7	2.3	2.1	1.9	2.0	1.7
CV	1.9	1.9	1.7	1.5	1.4	1.4	1.2
SEM	0.49	0.49	0.42	0.37	0.35	0.36	0.30

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	99	99	99	98	99	99
5	100	101	101	101	101	101	101
6	100	100	100	101	100	101	100
7	100	99	99	99	99	99	99
8	100	99	99	100	99	99	99
9	100	100	100	101	102	101	100
10	100	99	99	100	100	101	101
11	100	99	98	99	100	100	99
12	100	98	99	99	100	99	97
13	100	101	102	100	101	100	99
14	100	99	100	99	99	98	99
15	100	99	99	99	98	100	102
17	100	97	99	98	101	98	98
18	100	100	99	101	101	99	100
19	100	98	97	97	97	97	97
20	100	101	100	101	99	101	99
21	100	103	100	96	99	99	98
22	100	102	102	99	100	101	103
23	100	99	102	101	100	102	102
24	100	101	102	104	104	101	108
25	100	98	98	98	99	99	99
27	100	97	98	97	97	97	99
28	100	100	99	99	99	100	99
29	100	101	99	100	101	101	102
31	100	103	105	106	105	105	105
33	100	100	101	100	103	101	100
34	100	94	96	96	99	99	101
35	100	99	100	102	101	101	100
36	100	99	100	100	100	102	101
37	100	105	104	104	104	104	103
38	100	98	99	98	98	99	99
Mean	100	100	100	100	100	100	100
SD	0.0	2.1	2.0	2.3	2.0	1.8	2.2
CV	0.0	2.1	2.0	2.3	2.0	1.8	2.2

Serum Potassium (meq/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	4.5	4.5	4.5	4.5	4.4	4.4	4.5
5	3.9	4.4	4.1	4.2	4.4	4.4	4.6
6	4.4	4.3	4.2	3.9	4.3	3.8	4.0
7	4.2	4.2	4.3	4.2	4.8	4.2	3.6
8	4.2	4.3	4.3	4.1	4.7	4.3	4.4
9	4.0	4.1	4.1	4.6	3.9	3.8	3.8
10	4.4	4.4	4.2	4.5	4.2	4.2	4.3
11	4.5	4.0	4.7	4.4	5.6	5.0	4.9
12	4.3	3.7	3.7	3.9	4.0	3.9	3.6
13	4.0	4.2	4.0	4.3	4.1	4.1	4.6
14	3.8	4.1	4.0	4.4	4.3	3.7	4.4
15	4.3	4.0	4.2	5.0	3.8	4.4	4.0
17	3.9	4.2	4.2	4.3	4.1	4.3	4.1
18	3.7	4.7	4.5	4.4	4.4	4.8	5.4
19	4.1	4.2	4.5	5.0	4.4	4.5	4.6
20	4.0	4.0	4.1	4.1	3.9	4.5	3.9
21	3.6	4.2	3.9	4.0	4.2	4.0	4.1
22	4.2	3.8	3.9	3.8	4.2	3.7	3.7
23	4.1	3.8	7.7	3.9	4.0	4.5	4.0
24	4.0	4.6	5.0	4.6	4.9	4.7	4.4
25	4.5	4.3	4.4	4.5	4.3	4.2	4.4
27	5.0	4.6	4.2	4.7	4.7	4.7	5.0
28	4.3	3.8	4.0	4.3	4.3	4.2	4.7
29	4.0	4.0	5.2	4.6	4.6	4.7	4.2
31	4.2	4.7	4.1	3.9	4.3	4.3	3.5
33	4.4	4.2	4.4	4.4	4.0	4.1	4.3
34	4.4	4.9	4.7	4.7	4.7	4.8	4.6
35	3.8	3.6	4.0	4.0	3.8	3.6	3.8
36	4.2	5.4	5.0	4.7	4.6	4.3	3.8
37	4.7	4.7	4.4	4.3	4.6	4.7	4.6
38	4.3	3.8	4.1	4.0	4.4	4.1	3.9
Mean	4.2	4.2	4.4	4.3	4.4	4.3	4.2
SD	0.3	0.4	0.7	0.3	0.4	0.4	0.5
CV	7.1	9.2	15.9	7.5	8.6	8.4	10.6
SEM	0.05	0.07	0.13	0.06	0.07	0.06	0.08

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	100	100	100	98	98	100
5	100	113	105	108	113	113	118
6	100	98	95	89	98	86	91
7	100	100	102	100	114	100	86
8	100	102	102	98	112	102	105
9	100	103	103	115	98	95	95
10	100	100	95	102	95	95	98
11	100	89	104	98	124	111	109
12	100	86	86	91	93	91	84
13	100	105	100	108	103	103	115
14	100	108	105	116	113	97	116
15	100	93	98	116	88	102	93
17	100	108	108	110	105	110	105
18	100	127	122	119	119	130	146
19	100	102	110	122	107	110	112
20	100	100	103	103	98	113	98
21	100	117	108	111	117	111	114
22	100	90	93	90	100	88	88
23	100	93	188	95	98	110	98
24	100	115	125	115	123	118	110
25	100	96	98	100	96	93	98
27	100	92	84	94	94	94	100
28	100	88	93	100	100	98	109
29	100	100	130	115	115	118	105
31	100	112	98	93	102	102	83
33	100	95	100	100	91	93	98
34	100	111	107	107	107	109	105
35	100	95	105	105	100	95	100
36	100	129	119	112	110	102	90
37	100	100	94	91	98	100	98
38	100	88	95	93	102	95	91
Mean	100	102	106	104	104	103	102
SD	0.0	10.8	18.4	9.5	9.5	9.8	12.6
CV	0.0	10.6	17.4	9.2	9.1	9.6	12.4

T3 Resin Uptake (%) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0.37	0.39	0.38	0.37	0.36	0.36	0.40
5	0.47	0.40	0.39	0.42	0.43	0.41	0.43
6	0.38	0.39	0.38	0.39	0.39	0.40	0.37
7	0.36	0.37	0.36	0.37	0.39	0.38	0.46
8	0.40	0.37	0.42	0.37	0.36	0.32	0.39
9	0.39	0.39	0.39	0.41	0.40	0.40	0.41
10	0.40	0.43	0.46	0.51	0.45	0.47	0.46
11	0.43	0.49	0.43	0.48	0.45	0.43	0.53
12	0.43	0.41	0.44	0.46	0.42	0.41	0.39
13	0.39	0.43	0.41	0.49	0.43	0.44	0.46
14	0.37	0.37	0.37	0.42	0.34	0.37	0.43
15	0.37	0.39	0.41	0.41	0.34	0.37	0.40
17	0.39	0.37	0.39	0.38	0.37	0.38	0.41
18	0.42	0.42	0.39	0.40	0.34	0.40	0.40
19	0.49	0.46	0.46	0.40	0.35	0.45	0.44
20	0.43	0.42	0.41	0.36	0.45	0.44	0.43
21	0.39	0.35	0.37	0.41	0.44	0.41	0.38
22	0.36	0.33	0.43	0.36	0.38	0.39	0.39
23	0.40	0.41	0.39	0.40	0.45	0.45	0.44
24	0.42	0.37	0.38	0.34	0.39	0.39	0.41
25	0.43	0.39	0.45	0.50	0.52	0.49	0.46
27	0.43	0.41	0.40	0.42	0.45	0.41	0.41
28	0.41	0.43	0.42	0.44	0.42	0.38	0.40
29	0.43	0.44	0.44	0.43	0.48	0.54	0.48
31	0.47	0.43	0.44	0.40	0.41	0.37	0.40
33	0.46	0.46	0.45	0.44	0.42	0.41	0.41
34	0.44	0.43	0.44	0.45	0.46	0.42	0.44
35	0.44	0.46	0.44	0.48	0.43	0.43	0.43
36	0.56	0.50	0.51	0.52	0.55	0.54	0.51
37	0.57	0.38	0.55	0.36	0.36	0.37	0.38
38	0.42	0.40	0.37	0.36	0.35	0.39	0.36
Mean	0.42	0.41	0.42	0.42	0.41	0.41	0.42
SD	0.05	0.04	0.04	0.05	0.05	0.05	0.04
CV	11.9	9.6	10.1	11.6	12.7	11.7	9.3
SEM	0.009	0.007	0.008	0.009	0.009	0.009	0.007

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	105	103	100	97	97	108
5	100	85	83	89	91	87	91
6	100	103	100	103	103	105	97
7	100	103	100	103	108	106	128
8	100	93	105	93	90	80	98
9	100	100	100	105	103	103	105
10	100	108	115	128	113	118	115
11	100	114	100	107	105	100	123
12	100	95	102	107	98	95	91
13	100	110	105	126	110	113	118
14	100	100	100	114	92	100	116
15	100	105	111	111	92	100	108
17	100	95	100	97	95	97	105
18	100	100	93	95	81	95	95
19	100	94	94	82	71	92	90
20	100	98	95	84	105	102	100
21	100	90	95	105	113	105	97
22	100	92	119	100	108	108	108
23	100	103	98	100	113	113	110
24	100	88	90	81	93	93	98
25	100	91	105	116	121	114	107
27	100	95	93	98	105	95	95
28	100	105	102	107	102	93	98
29	100	102	102	100	112	126	112
31	100	91	94	85	87	79	85
33	100	100	98	96	91	89	89
34	100	98	100	102	105	95	100
35	100	105	100	109	98	98	98
36	100	89	91	93	98	96	91
37	100	67	96	63	63	65	67
38	100	95	88	86	83	93	86
Mean	100	97	99	99	98	98	101
SD	0.0	8.9	7.4	13.3	12.5	11.9	12.5
CV	0.0	9.2	7.5	13.4	12.8	12.1	12.4

Free T4 Index of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	0.30	0.41	0.40	0.40	0.39	0.24	0.21
5	0.41	0.52	0.50	0.53	0.54	0.55	0.48
6	0.45	0.53	0.54	0.61	0.63	0.57	0.55
7	0.37	0.37	0.37	0.35	0.48	0.48	0.42
8	0.50	0.43	0.42	0.48	0.45	0.43	0.45
9	0.47	0.28	0.53	0.57	0.60	0.57	0.48
10	0.44	0.55	0.49	0.66	0.52	0.59	0.55
11	0.28	0.46	0.38	0.46	0.45	0.48	0.50
12	0.40	0.42	0.38	0.44	0.52	0.45	0.28
13	0.47	0.66	0.60	0.79	0.74	0.75	0.75
14	0.37	0.42	0.48	0.52	0.48	0.43	0.45
15	0.46	0.55	0.48	0.52	0.37	0.45	0.30
17	0.38	0.39	0.43	0.49	0.48	0.45	0.54
18	0.55	0.56	0.52	0.60	0.55	0.50	0.50
19	0.33	0.34	0.21	0.19	0.20	0.24	0.20
20	0.47	0.65	0.49	0.55	0.66	0.54	0.66
21	0.43	0.51	0.60	0.49	0.56	0.53	0.50
22	0.33	0.44	0.43	0.35	0.39	0.39	0.41
23	0.48	0.55	0.54	0.54	0.54	0.45	0.51
24	0.47	0.45	0.23	0.47	0.17	0.12	0.15
25	0.30	0.41	0.40	0.38	0.37	0.47	0.50
27	0.49	0.55	0.55	0.52	0.63	0.63	0.64
28	0.42	0.49	0.49	0.47	0.51	0.58	0.62
29	0.39	0.52	0.49	0.47	0.56	0.63	0.57
31	0.48	0.47	0.42	0.41	0.40	0.37	0.34
33	0.46	0.45	0.53	0.63	0.60	0.56	0.55
34	0.50	0.54	0.53	0.59	0.60	0.49	0.54
35	0.40	0.54	0.54	0.60	0.52	0.54	0.62
36	0.43	0.44	0.46	0.48	0.50	0.51	0.48
37	0.98	0.35	0.41	0.34	0.40	0.42	0.47
38	0.48	0.54	0.55	0.54	0.55	0.66	0.55
Mean	0.44	0.48	0.46	0.50	0.50	0.49	0.48
SD	0.12	0.09	0.09	0.11	0.12	0.13	0.14
CV	27.2	18.2	19.6	22.8	24.6	26.2	28.9
SEM	0.022	0.016	0.016	0.020	0.022	0.023	0.025

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	137	133	133	130	80	70
5	100	127	122	129	132	134	117
6	100	118	120	136	140	127	122
7	100	100	100	95	130	130	114
8	100	86	84	96	90	86	90
9	100	60	113	121	128	121	102
10	100	125	111	150	118	134	125
11	100	164	136	164	161	171	179
12	100	105	95	110	130	113	70
13	100	140	128	168	157	160	160
14	100	114	130	141	130	116	122
15	100	120	104	113	80	98	65
17	100	103	113	129	126	118	142
18	100	102	95	109	100	91	91
19	100	103	64	58	61	73	61
20	100	138	104	117	140	115	140
21	100	119	140	114	130	123	116
22	100	133	130	106	118	118	124
23	100	115	113	113	113	94	106
24	100	96	49	100	36	26	32
25	100	137	133	127	123	157	167
27	100	112	112	106	129	129	131
28	100	117	117	112	121	138	148
29	100	133	126	121	144	162	146
31	100	98	88	85	83	77	71
33	100	98	115	137	130	122	120
34	100	108	106	118	120	98	108
35	100	135	135	150	130	135	155
36	100	102	107	112	116	119	112
37	100	36	42	35	41	43	48
38	100	113	115	113	115	138	115
Mean	100	113	109	117	116	114	112
SD	0.0	24.4	24.0	27.1	29.5	32.7	35.7
CV	0.0	21.7	22.0	23.2	25.4	28.6	32.0

Total T4 (nmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	81	104	105	107	108	68	53
5	88	130	127	127	125	133	122
6	118	136	141	156	162	142	148
7	95	99	102	95	124	125	93
8	125	120	123	129	126	136	107
9	120	72	136	138	150	142	117
10	109	127	160	129	115	125	119
11	66	94	89	101	99	111	94
12	93	103	86	95	123	110	71
13	120	154	147	162	175	171	164
14	101	113	130	124	140	117	103
15	125	142	118	126	94	121	76
17	97	104	110	130	130	116	134
18	130	133	133	151	140	125	124
19	68	75	46	48	57	63	48
20	110	154	118	152	174	124	155
21	111	145	163	120	127	128	130
22	91	133	126	97	101	99	105
23	120	134	138	135	119	101	117
24	112	122	60	138	47	30	38
25	71	104	84	76	70	96	109
27	115	135	137	126	139	154	159
28	102	114	118	107	120	154	156
29	90	116	112	109	116	118	119
31	100	108	95	101	97	101	84
33	101	98	118	144	142	137	135
34	115	126	120	130	130	116	124
35	91	118	125	125	123	128	144
36	76	88	91	93	90	95	93
37	173	94	73	95	111	114	125
38	114	133	148	153	156	168	154
Mean	104	117	115	120	120	118	114
SD	21.6	21.2	27.6	25.5	29.9	29.9	33.1
CV	20.8	18.1	23.9	21.3	24.9	25.4	29.1
SEM	3.88	3.80	4.96	4.58	5.37	5.37	5.94

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	128	130	132	133	84	65
5	100	148	144	144	142	151	139
6	100	115	119	132	137	120	125
7	100	104	107	100	131	132	98
8	100	96	98	103	101	106	86
9	100	60	113	115	125	118	98
10	100	117	147	118	106	115	109
11	100	142	135	153	150	168	142
12	100	111	92	102	132	118	76
13	100	128	123	135	146	143	137
14	100	112	129	123	139	116	102
15	100	114	94	101	75	97	61
17	100	107	113	134	134	120	138
18	100	102	102	116	108	96	95
19	100	110	68	71	84	78	71
20	100	140	107	138	158	113	141
21	100	131	147	108	114	115	117
22	100	146	138	107	111	109	115
23	100	112	115	113	99	84	98
24	100	109	54	123	42	27	33
25	100	146	118	107	99	135	154
27	100	117	119	110	121	134	138
28	100	112	116	105	118	151	153
29	100	129	124	121	129	131	132
31	100	108	95	101	97	101	84
33	100	98	117	143	141	136	134
34	100	110	104	113	113	101	108
35	100	130	137	137	135	141	158
36	100	116	120	122	118	125	122
37	100	54	42	55	64	66	72
38	100	117	130	134	137	147	135
Mean	100	115	113	117	117	115	111
SD	0.0	21.1	24.7	20.7	26.1	28.6	31.5
CV	0.0	18.3	21.9	17.7	22.3	24.8	28.4

T3 (nmol/L) of Patients Taking Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	2.20	1.86	1.86	1.86	2.20	1.80	1.95
5	1.45	1.92	1.80	1.75	1.75	1.85	1.50
6	1.50	1.72	1.90	1.59	1.85	1.70	2.20
7	1.60	1.64	1.70	1.60	2.00	1.60	1.25
8	2.00	2.20	2.35	2.10	2.35	2.30	2.30
9	2.02	1.10	1.85	1.60	1.55	1.80	1.45
10	1.45	2.05	2.35	2.06	1.80	2.45	2.15
11	1.90	1.70	1.67	1.70	1.65	1.74	1.96
12	1.60	1.30	1.65	1.45	1.55	1.90	1.50
13	1.80	2.20	2.30	2.00	1.76	1.68	1.70
14	1.65	2.10	1.50	2.00	2.10	1.76	1.75
15	1.75	1.84	1.56	2.00	1.64	1.85	2.60
17	1.95	1.67	1.60	1.60	1.75	1.90	1.85
18	1.65	1.70	1.93	1.88	1.95	2.20	1.90
19	1.37	1.30	1.20	1.18	1.65	1.80	1.90
20	2.05	2.05	2.04	2.05	1.89	1.74	2.00
21	2.20	2.20	2.80	2.03	1.78	2.15	2.20
22	2.00	2.40	2.25	0.35	2.30	2.40	2.50
23	2.38	2.10	1.80	1.90	1.78	1.80	2.10
24	1.50	1.85	1.24	1.70	1.60	1.62	1.40
25	1.77	2.20	2.08	1.96	2.00	2.35	1.90
27	1.80	1.76	1.80	1.90	1.50	2.15	1.52
28	2.15	1.75	1.90	1.87	2.00	2.05	1.45
29	1.36	1.70	2.20	1.81	1.50	1.20	1.40
31	1.23	1.40	1.66	2.25	2.02	2.00	1.40
33	2.10	2.25	2.00	1.90	1.95	2.10	1.75
34	1.58	1.60	1.84	1.66	1.70	1.60	1.45
35	1.80	1.78	2.10	2.15	2.18	1.75	2.06
36	1.76	2.20	1.97	1.74	2.00	2.45	1.46
37	3.10	2.70	1.88	2.05	1.85	2.00	1.68
38	2.00	2.34	2.25	2.45	2.05	1.70	2.36
Mean	1.83	1.89	1.90	1.81	1.86	1.92	1.83
SD	0.37	0.36	0.33	0.37	0.23	0.29	0.36
CV	20.1	18.9	17.6	20.4	12.4	15.1	19.9
SEM	0.066	0.064	0.060	0.066	0.041	0.052	0.065

Percent Value of Parameter vs Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	100	85	85	85	100	82	89
5	100	132	124	121	121	128	103
6	100	109	120	101	117	108	139
7	100	100	104	98	122	98	76
8	100	110	118	105	118	115	115
9	100	54	92	79	77	89	72
10	100	141	162	142	124	169	148
11	100	89	88	89	87	92	103
12	100	81	103	91	97	119	94
13	100	122	128	111	98	93	94
14	100	127	91	121	127	107	106
15	100	105	89	114	94	106	149
17	100	86	82	82	90	97	95
18	100	103	117	114	118	133	115
19	100	95	88	86	120	131	139
20	100	100	100	100	92	85	98
21	100	100	127	92	81	98	100
22	100	120	113	18	115	120	125
23	100	88	76	80	75	76	88
24	100	123	83	113	107	108	93
25	100	124	118	111	113	133	107
27	100	98	100	106	83	119	84
28	100	81	88	87	93	95	67
29	100	125	162	133	110	88	103
31	100	114	135	183	164	163	114
33	100	107	95	90	93	100	83
34	100	103	119	107	110	103	94
35	100	99	117	119	121	97	116
36	100	125	112	99	114	139	83
37	100	87	61	66	60	65	54
38	100	117	113	123	103	85	118
Mean	100	105	107	102	105	108	102
SD	0.0	18.7	22.9	27.3	20.2	23.7	22.6
CV	0.0	17.8	21.5	26.7	19.3	22.0	22.1

SOD Activity (ng/mg-Hb) of Patients on Amiodarone

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4	ND						
5	548	554	554	773	556	530	580
6	694	745	785	717	536	612	547
7	585	539	576	720	771	635	590
8	565	707	750	636	579	710	721
9	604	616	549	774	592	543	696
10	550	718	525	578	568	533	523
11	494	529	603	562	652	702	372
12	477	551	782	567	638	509	528
13	651	568	650	494	464	396	758
14	789	397	710	530	501	496	600
15	667	551	583	513	643	647	529
17	581	255	707	485	326	404	484
18	386	826	466	339	452	495	456
19	701	632	474	233	506	500	601
20	489	513	444	617	484	560	579
21	318	445	361	410	582	596	527
22	253	442	399	439	735	340	306
23	333	325	325	394	495	512	580
24	343	362	400	395	467	592	527
25	440	441	442	354	392	484	454
27	474	480	631	578	479	518	516
28	409	729	538	531	400	417	493
29	543	661	566	527	392	484	454
31	448	570	570	480	479	518	516
33	597	611	482	594	502	421	415
34	496	502	606	478	492	553	498
35	592	465	507	523	618	477	435
36	618	584	583	602	510	426	439
37	513	423	574	598	575	521	481
38	504	719	687	558	597	506	447
Mean	522	548	561	532	533	521	520
SD	123	134	120	126	100	87	95
CV	23.6	24.4	21.3	23.7	18.7	16.6	18.2
SEM	22.5	24.4	21.9	23.0	18.2	15.8	17.3

Percent Value of Parameter vs. Baseline

Pt. ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
4							
5	100	101	101	141	101	97	106
6	100	107	113	103	77	88	79
7	100	92	98	123	132	109	101
8	100	125	133	113	102	126	128
9	100	102	91	128	98	90	108
10	100	131	95	105	103	97	95
11	100	107	122	114	132	142	75
12	100	116	164	119	134	107	111
13	100	87	100	76	71	61	116
14	100	50	90	67	63	63	76
15	100	83	87	77	96	97	79
17	100	44	122	78	56	70	83
18	100	214	121	88	117	128	118
19	100	90	68	33	72	71	86
20	100	105	91	126	99	115	118
21	100	140	114	129	183	187	166
22	100	175	158	174	291	134	121
23	100	98	98	118	149	154	174
24	100	106	117	115	136	173	154
25	100	100	100	81	89	110	103
27	100	101	133	122	101	109	109
28	100	178	132	130	98	102	121
29	100	122	104	97	72	89	84
31	100	127	127	103	107	116	115
33	100	102	81	99	84	71	70
34	100	101	122	96	99	111	100
35	100	79	86	88	104	81	74
36	100	91	94	97	83	69	71
37	100	82	112	117	112	101	94
38	100	143	136	111	118	100	89
Mean	100	110	110	106	109	106	104
SD	0.0	34.9	22.2	26.2	43.6	30.8	26.8
CV	0.0	31.8	20.1	24.8	39.9	29.1	25.7

SOD Activity (ng/mg-Hb) of Healthy Volunteers

Vol. ID	0 mo.	1 mo.	2 mo.	3 mo.	12 mo.	Intrasubject Variation
1	388	383	408	415	464	7.82
2	440	382	401	376	400	6.25
3	382	383	525	436	456	13.58
4	395	325	328	434	380	12.44
5	341	367	395	403	409	7.43
6	370	407	395	448	488	11.06
Mean	386	375	409	419	433	
SD	33	27	64	26	42	
CV	8.4	7.3	15.7	6.3	9.9	
SEM	13.3	11.2	26.1	10.8	17.3	

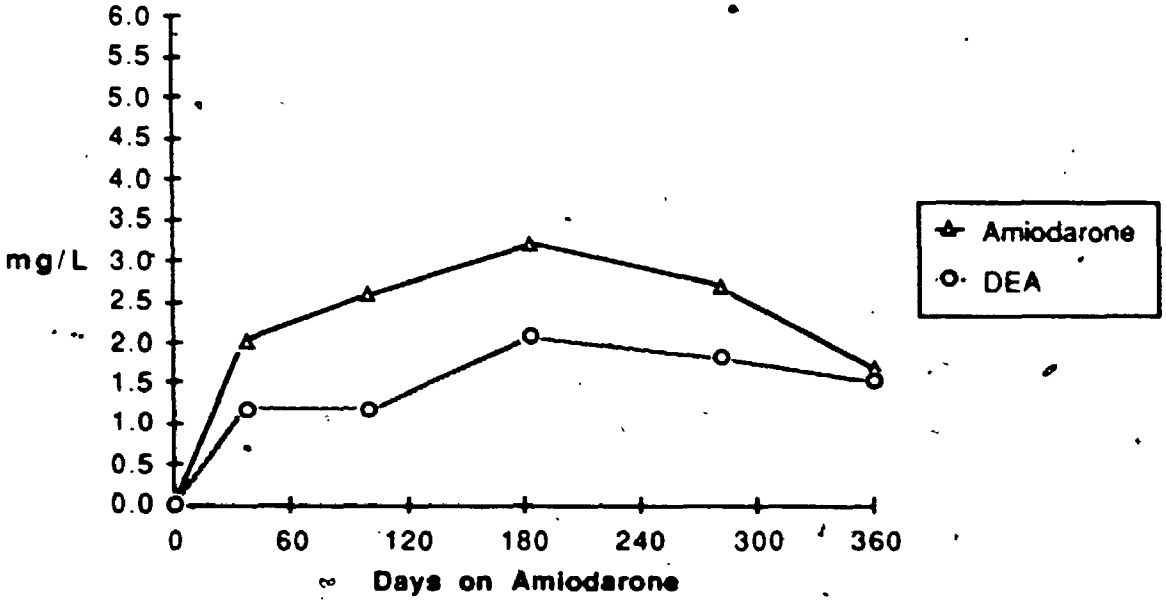
Percent Value of Parameter vs Baseline

Vol. ID	0 mo.	1 mo.	2 mo.	3 mo.	12 mo.
1	100	99	105	107	120
2	100	87	91	85	91
3	100	100	137	114	119
4	100	82	83	110	96
5	100	108	116	118	120
6	100	110	107	121	132
Mean	100	98	107	109	113
SD	0.0	11.1	19.1	12.8	15.9
CV	0.0	11.3	18.0	11.7	14.0

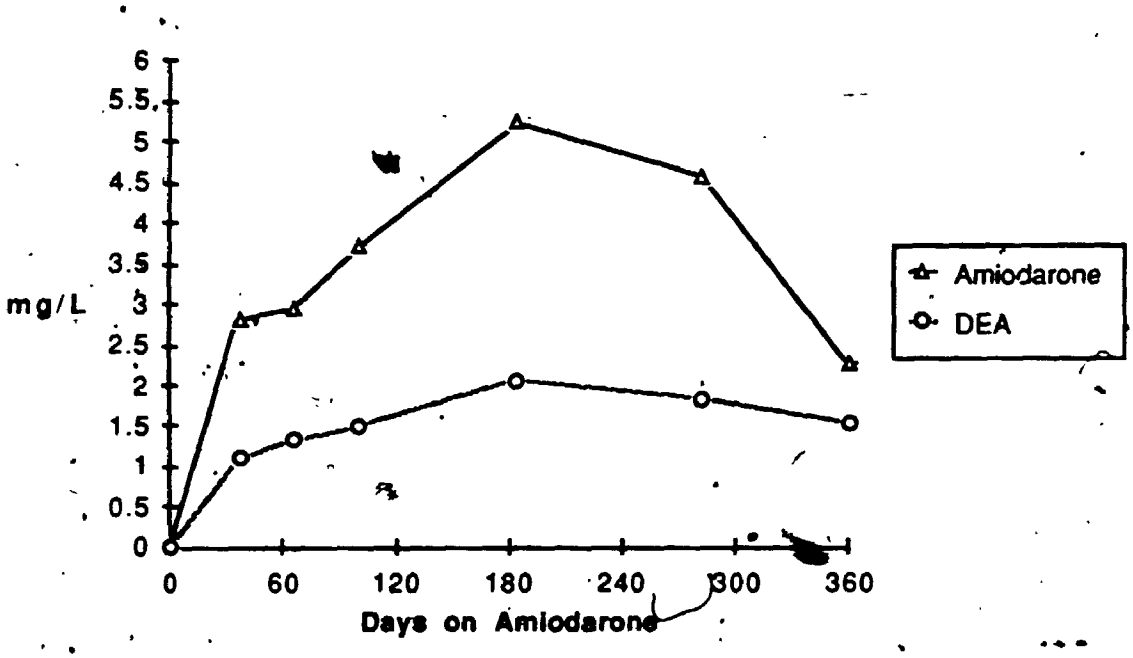
APPENDIX 6.

Amiodarone and desethylamiodarone concentration-time curves for patients in the study

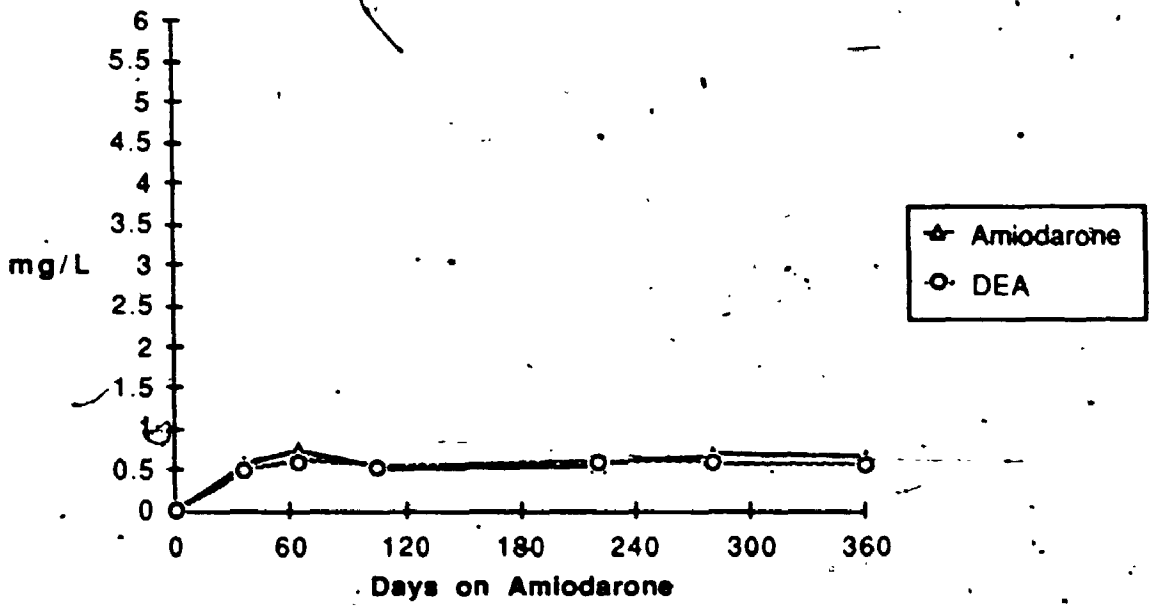
Patient 4 - Amiodarone and Desethylamiodarone Concentrations



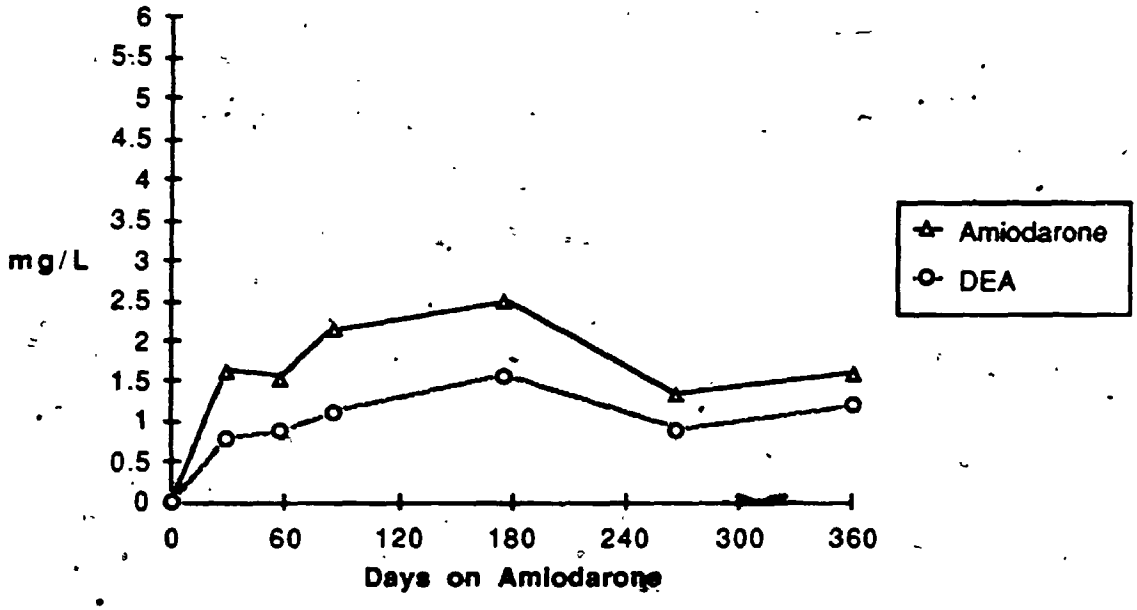
Patient 5 - Amiodarone and Desethylamiodarone Concentrations



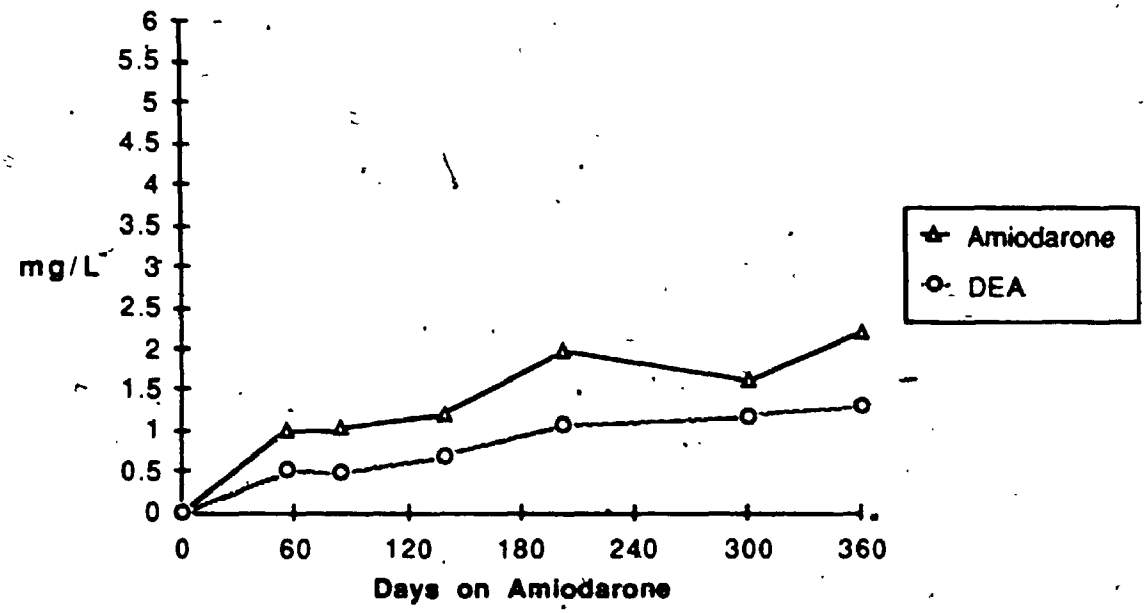
Patient 6 - Amiodarone and Desethylamiodarone Concentrations



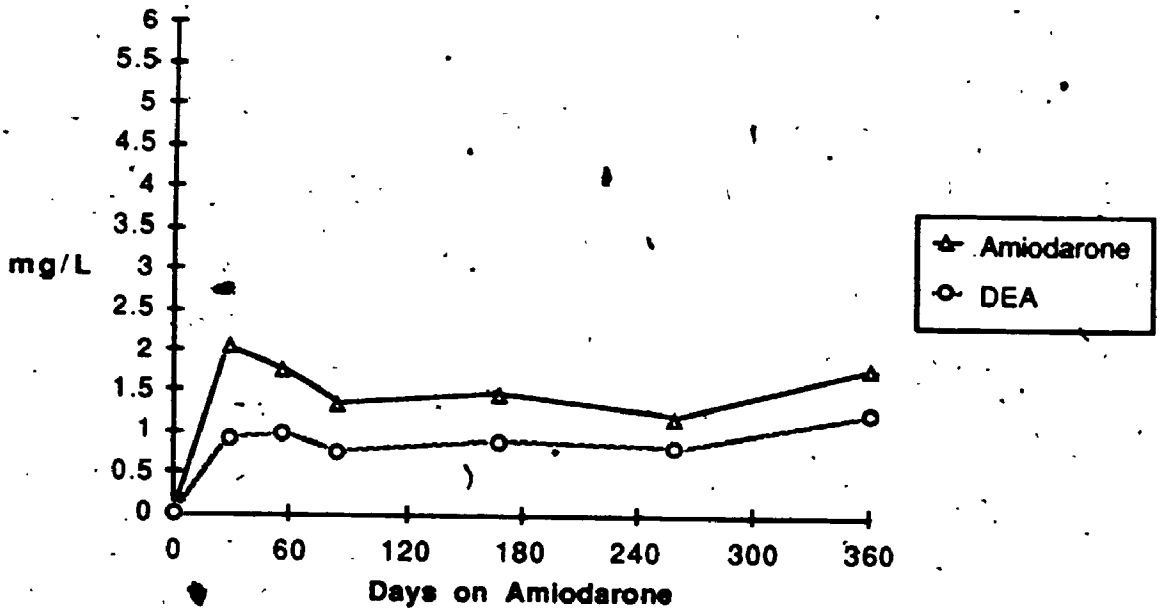
Patient 7 - Amiodarone and Desethylamiodarone Concentrations



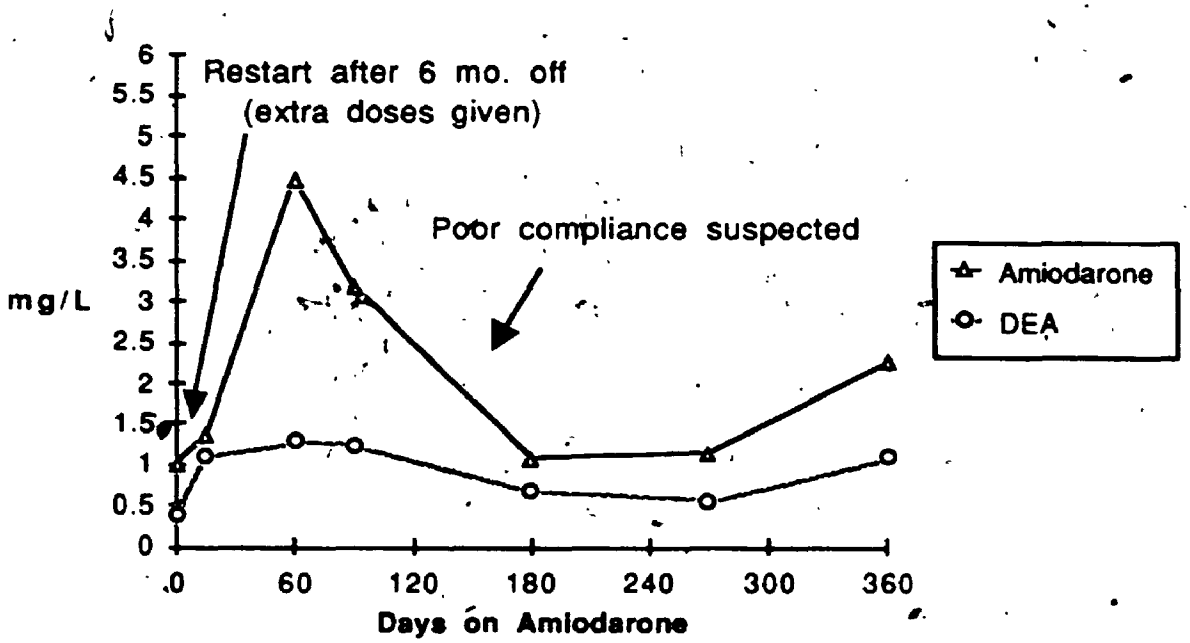
Patient 8 - Amiodarone and Desethylamiodarone Concentrations



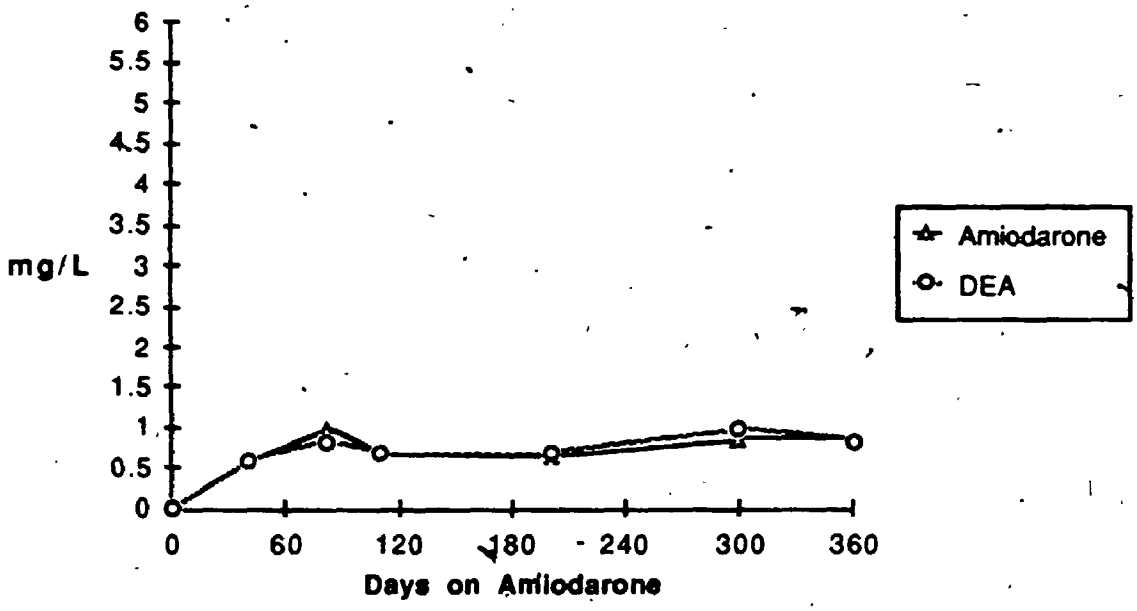
Patient 9 - Amiodarone and Desethylamiodarone Concentrations



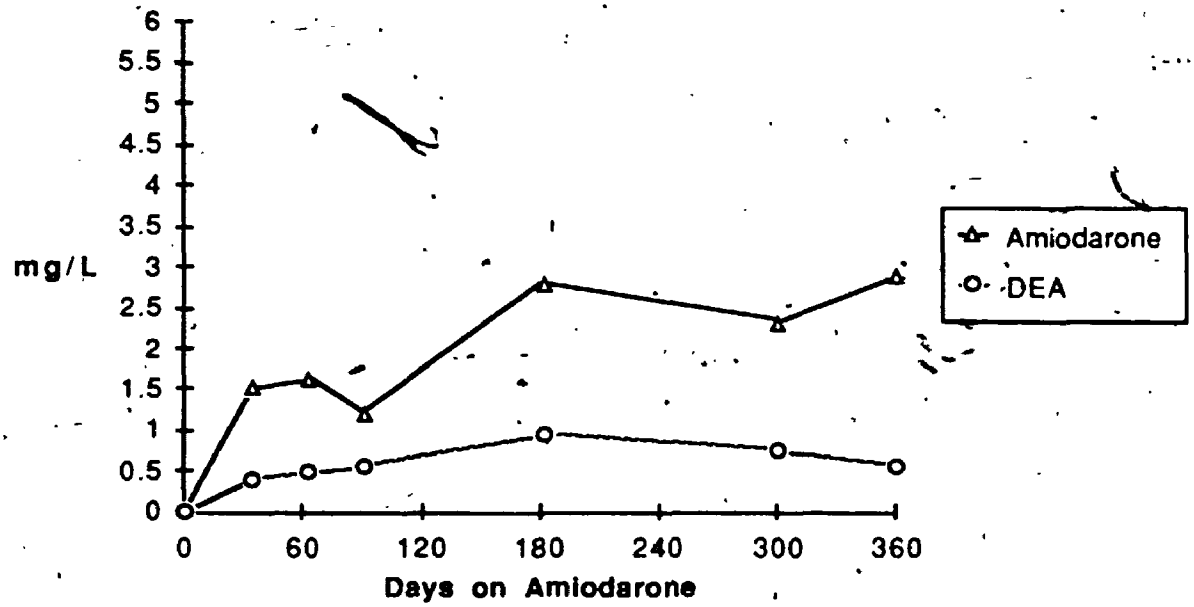
Patient 10 - Amiodarone and Desethylamiodarone Concentrations



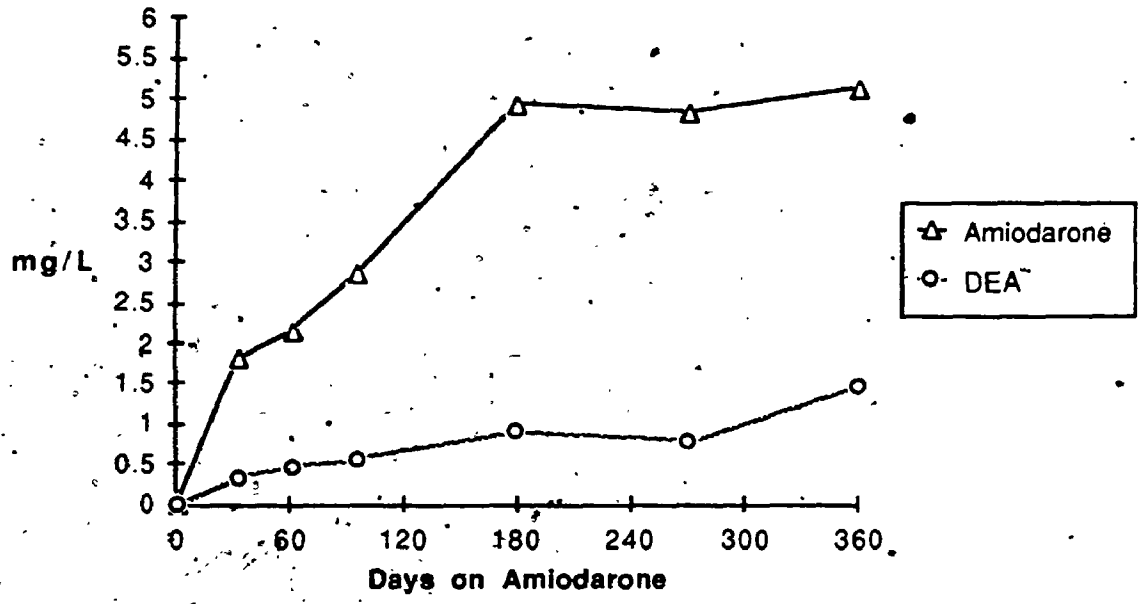
Patient 11 - Amiodarone and Desethylamiodarone Concentrations



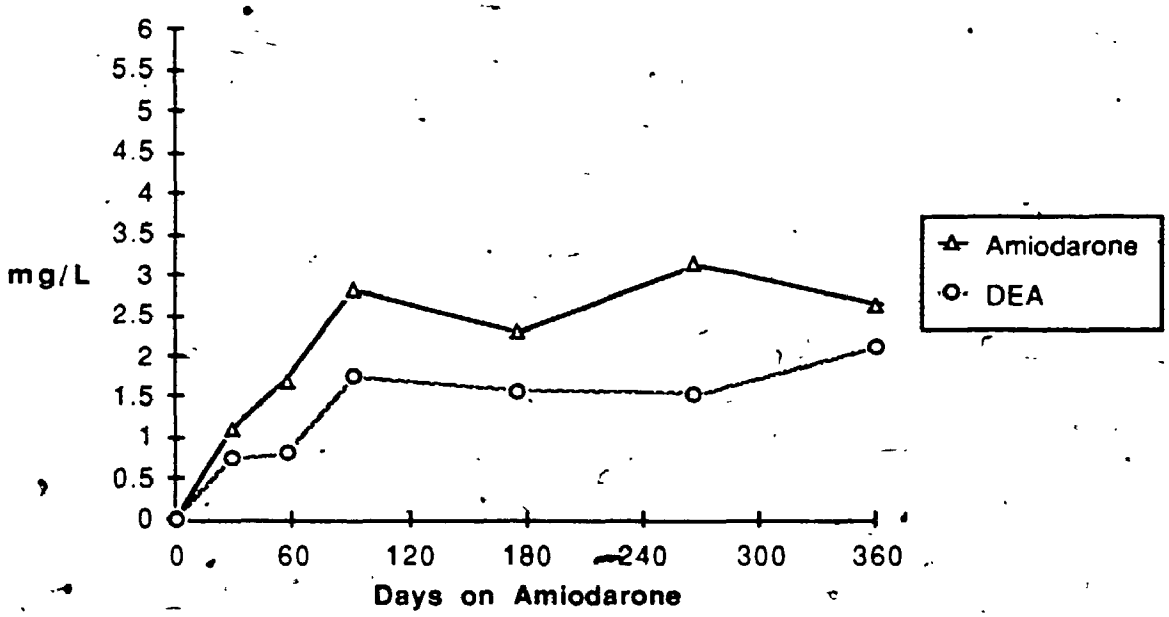
Patient 12 - Amiodarone and Desethylamiodarone Concentrations



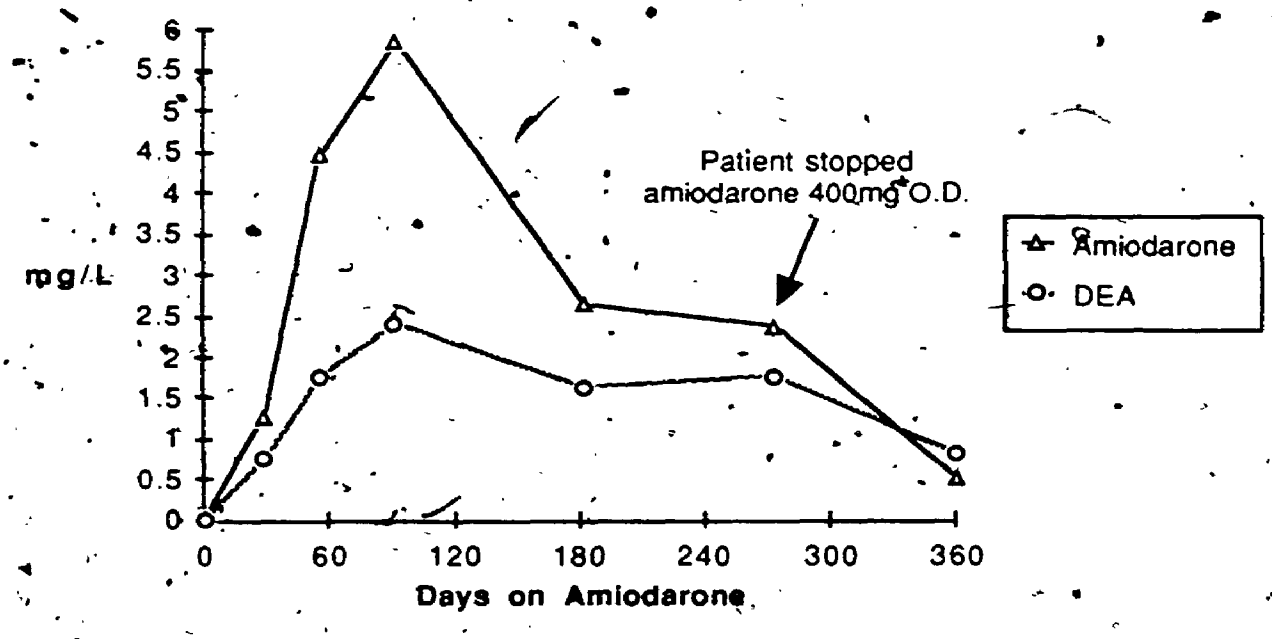
Patient 13 - Amiodarone and Desethylamiodarone Concentrations



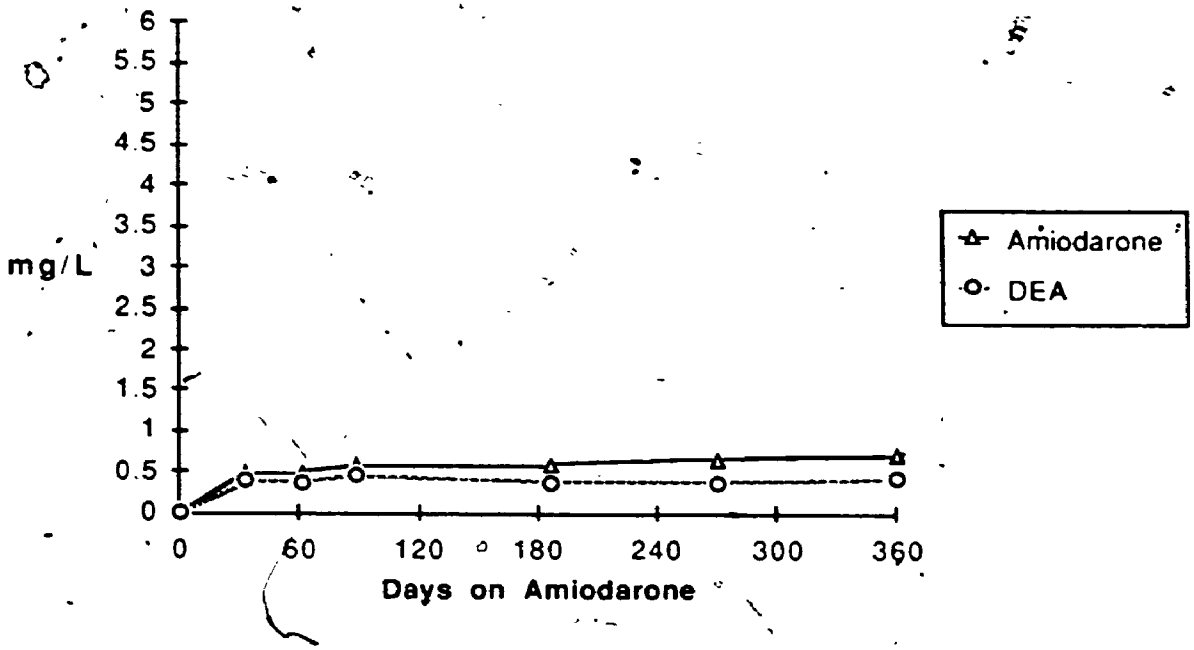
Patient 14 - Amiodarone and Desethylamiodarone Concentrations



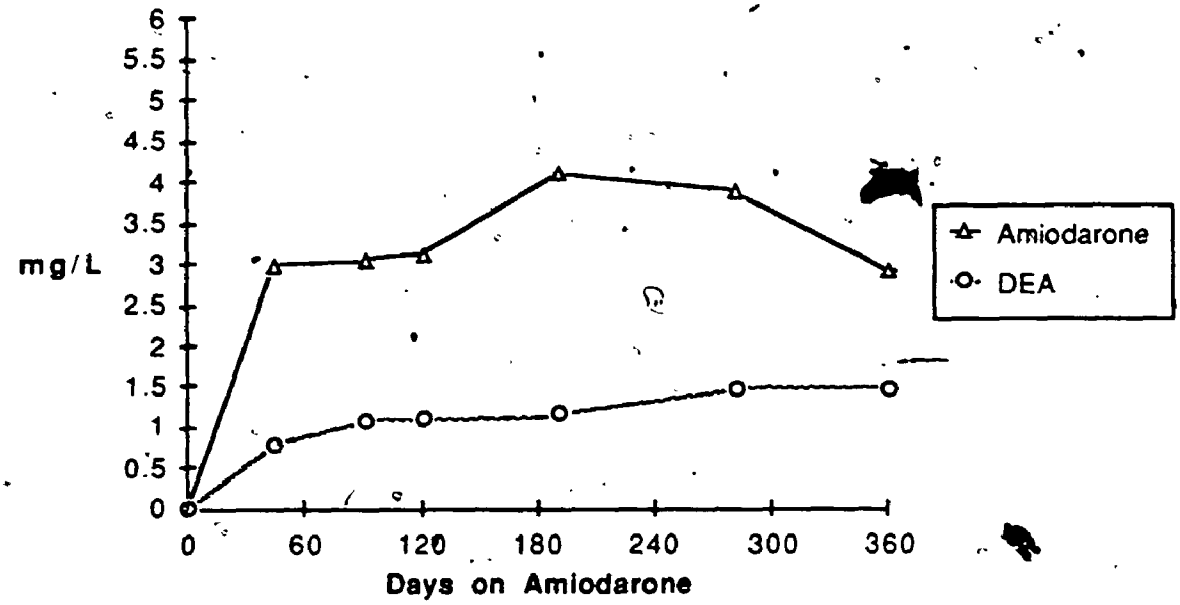
Patient 15 - Amiodarone and Desethylamiodarone Concentrations



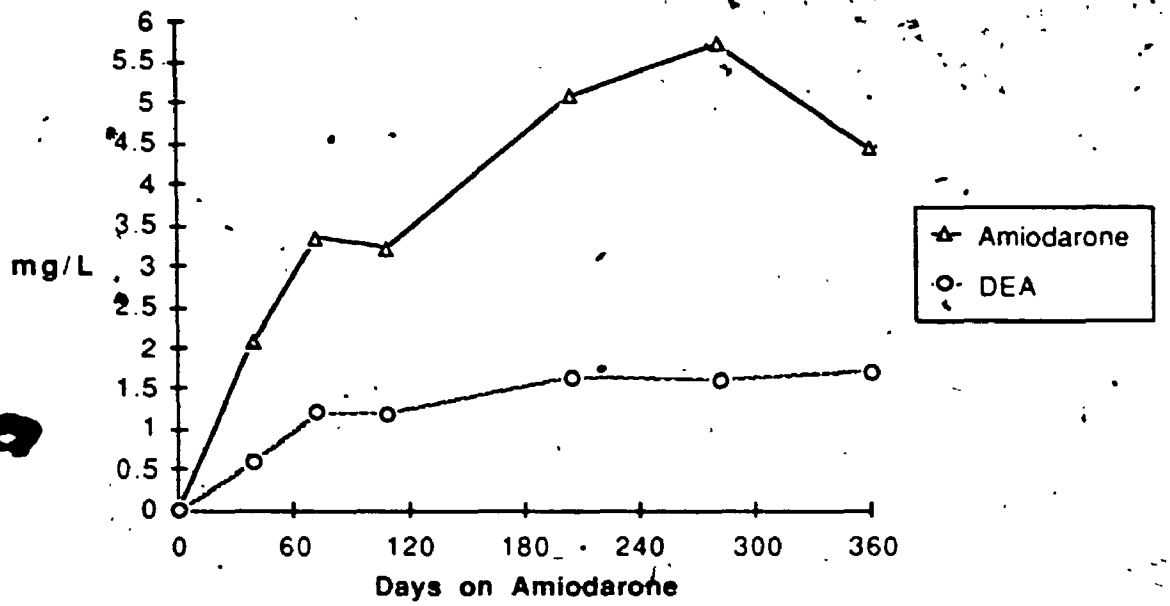
Patient 17 - Amiodarone and Desethylamiodarone Concentrations



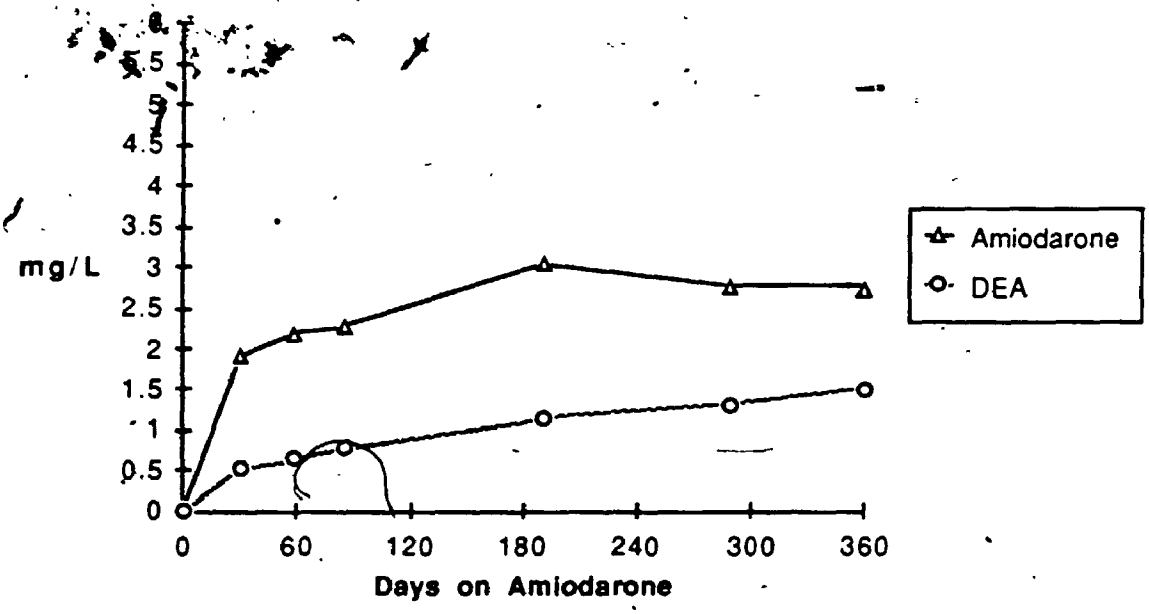
Patient 18 - Amiodarone and Desethylamiodarone Concentrations



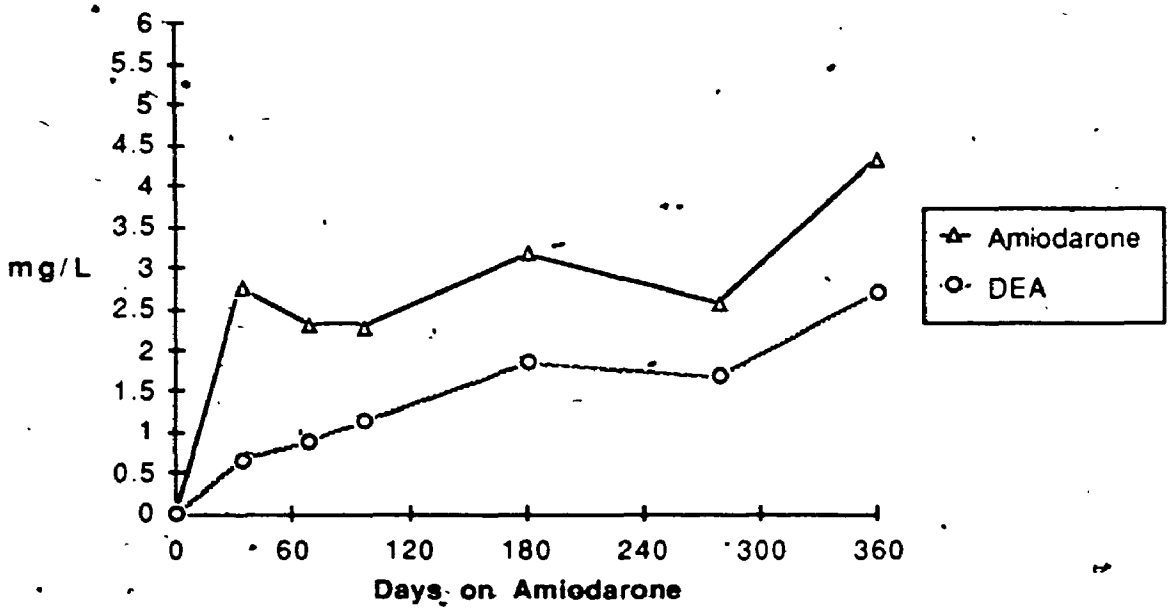
Patient 19 - Amiodarone and Desethylamiodarone Concentrations



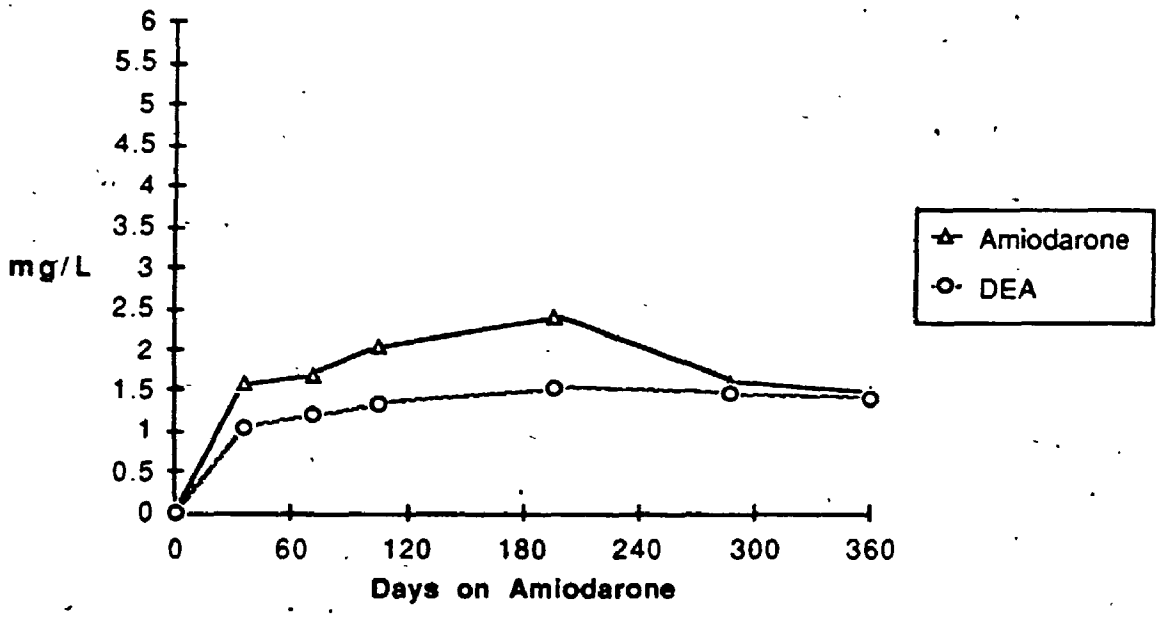
Patient 20 - Amiodarone and Desethylamiodarone Concentrations



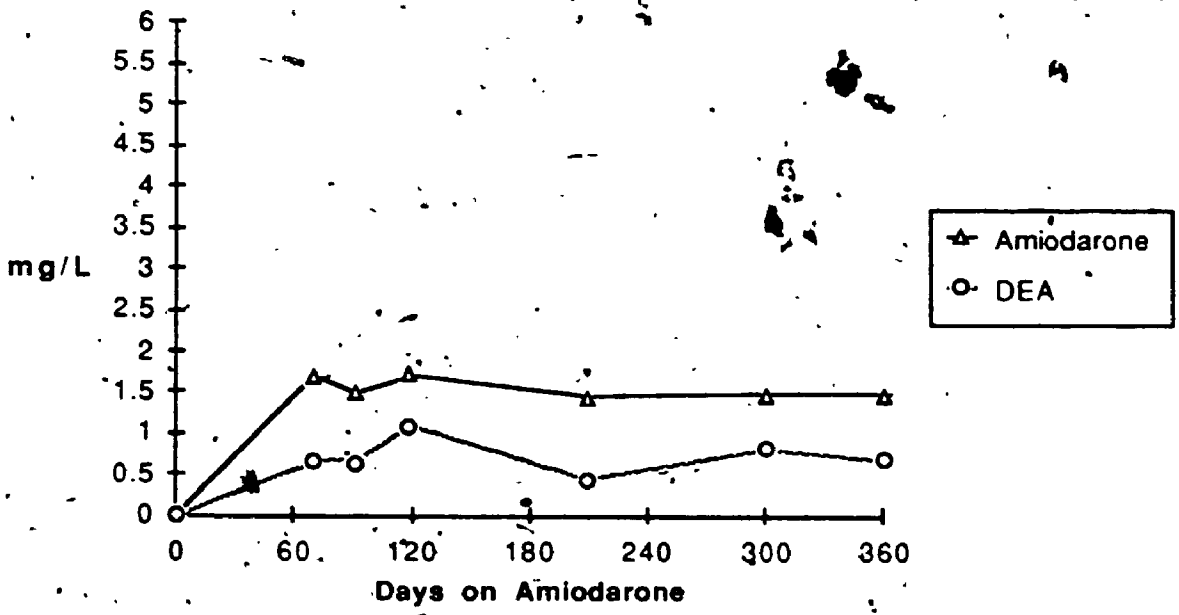
Patient 21 - Amiodarone and Desethylamiodarone Concentrations



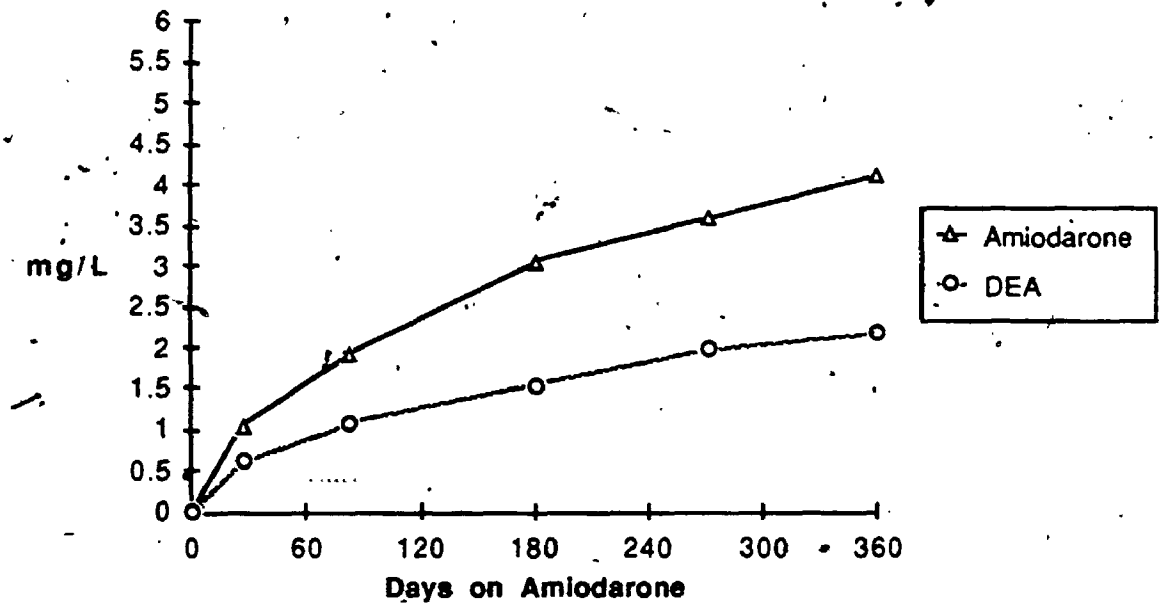
Patient 23 - Amiodarone and Desethylamiodarone Concentrations



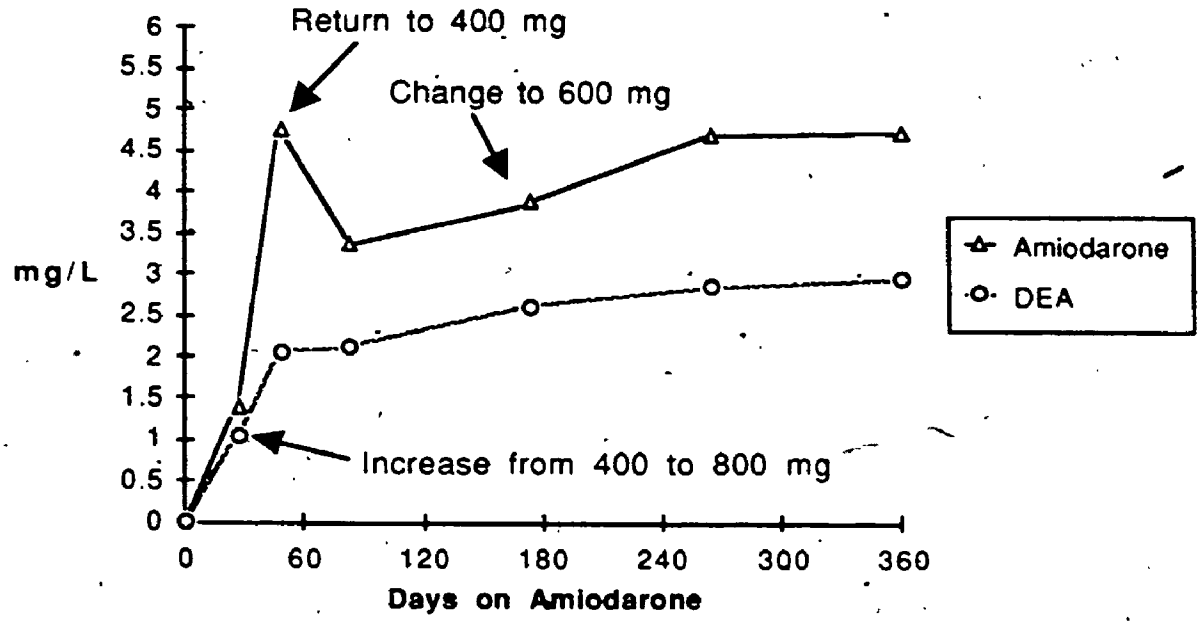
Patient 24 - Amiodarone and Desethylamiodarone Concentrations



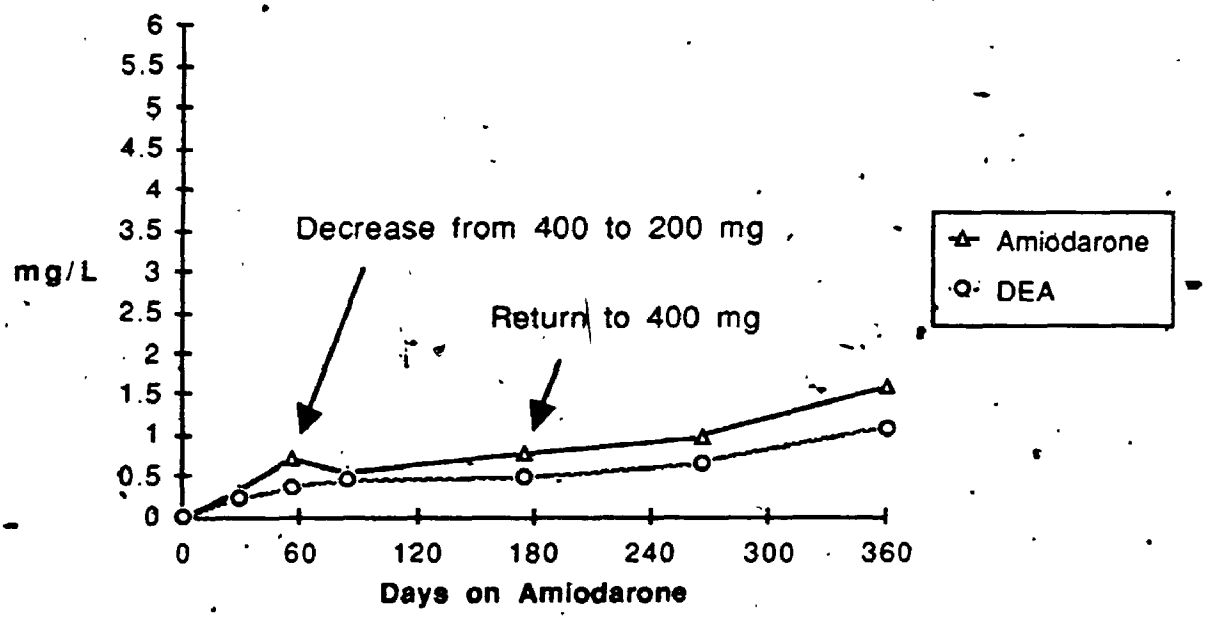
Patient 25 - Amiodarone and Desethylamiodarone Concentrations



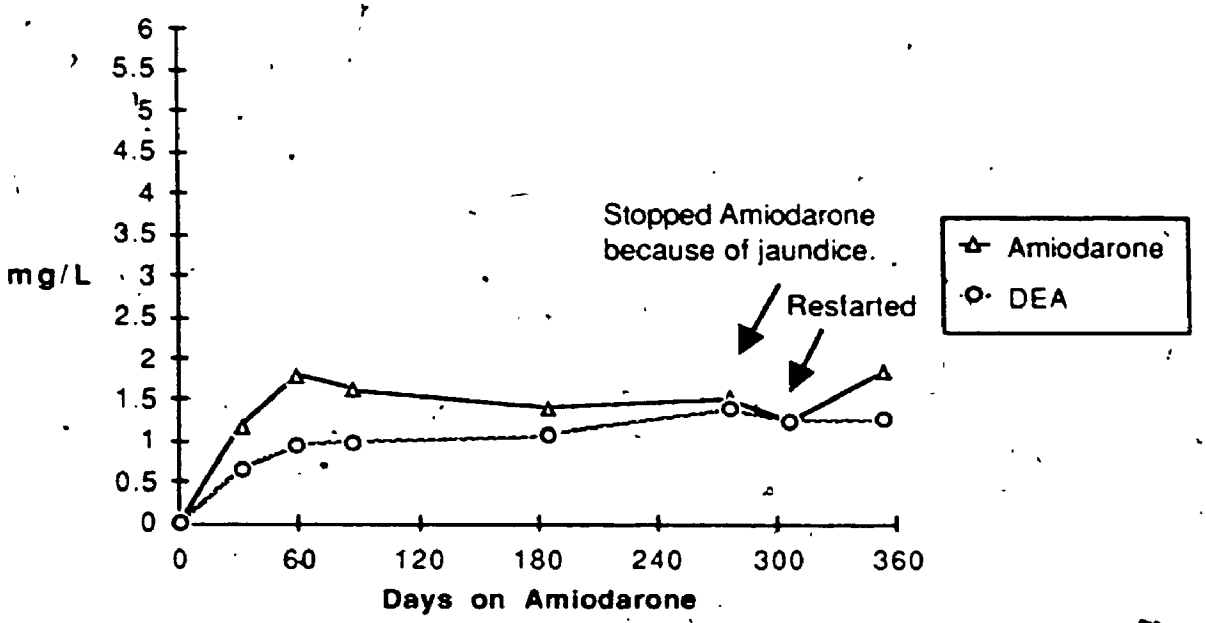
Patient 27 - Amiodarone and Desethylamiodarone Concentrations



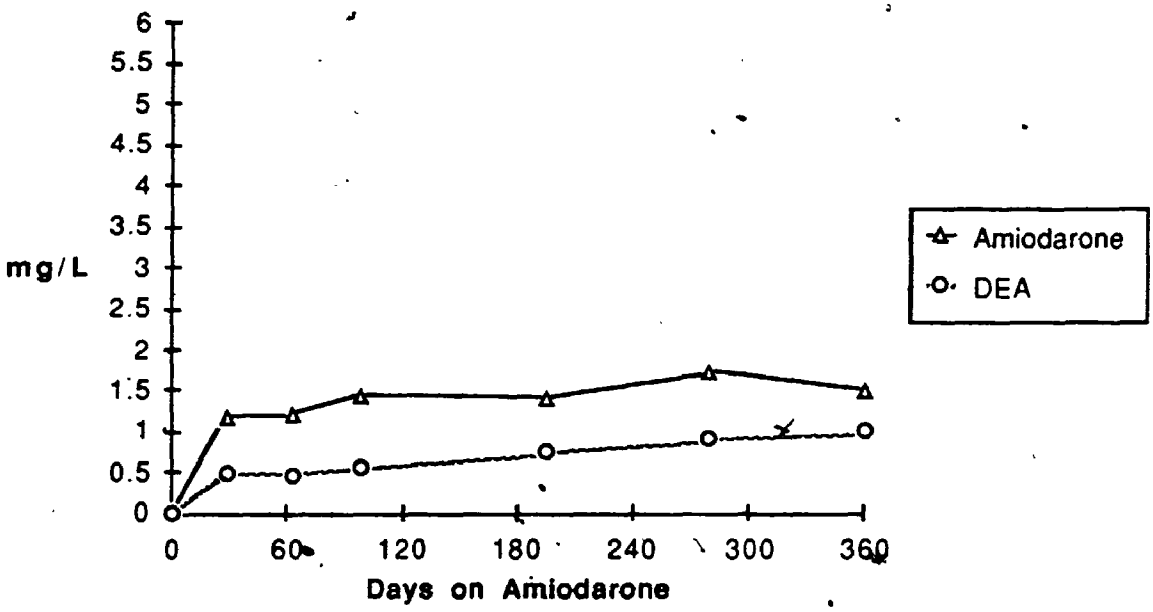
Patient 28 - Amiodarone and Desethylamiodarone Concentrations



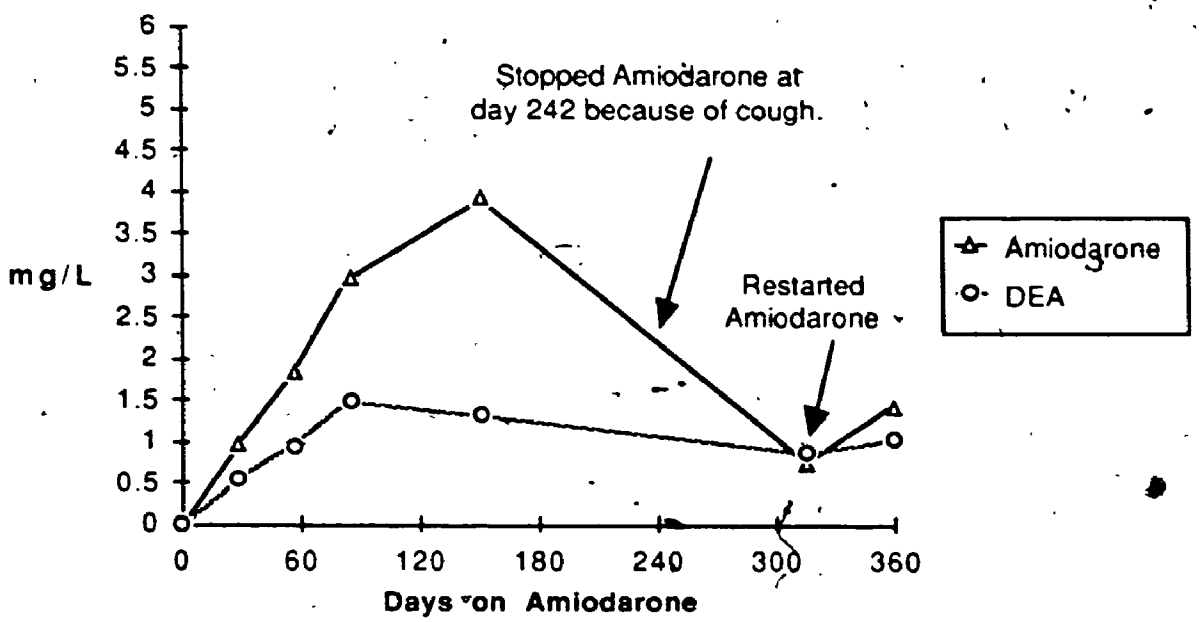
Patient 29 - Amiodarone and Desethylamiodarone Concentrations



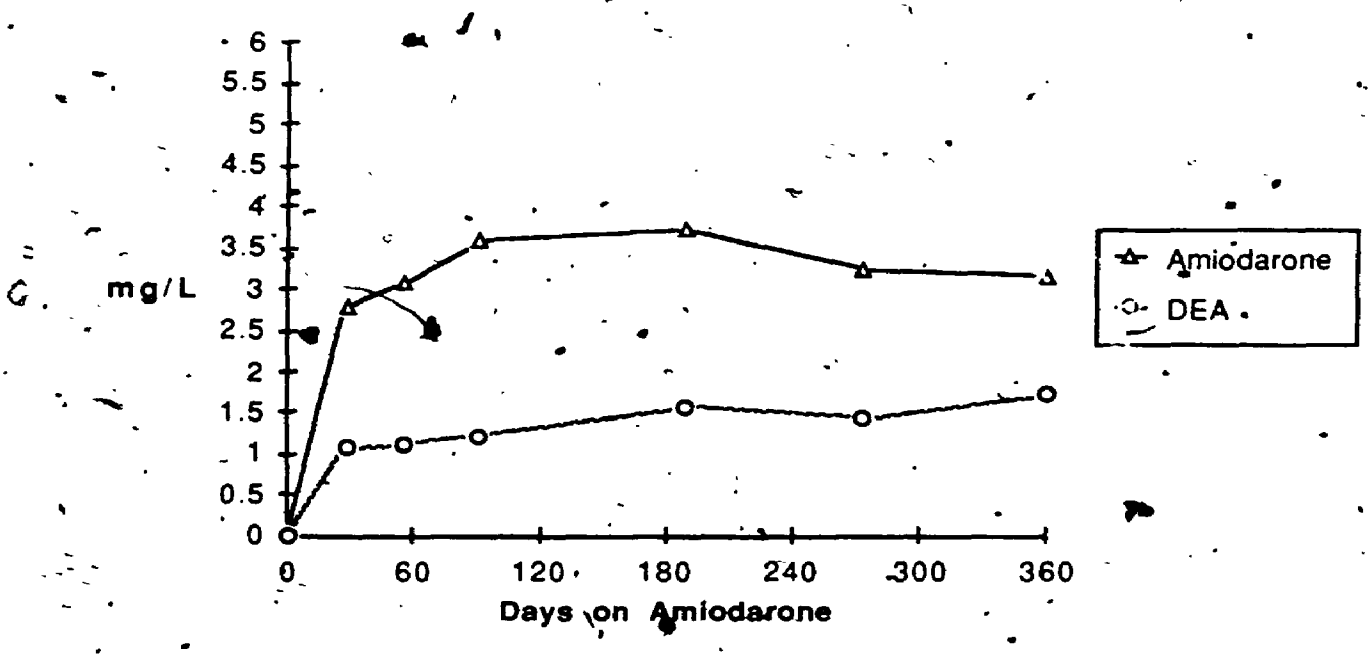
Patient 31- Amiodarone and Desethylamiodarone Concentrations



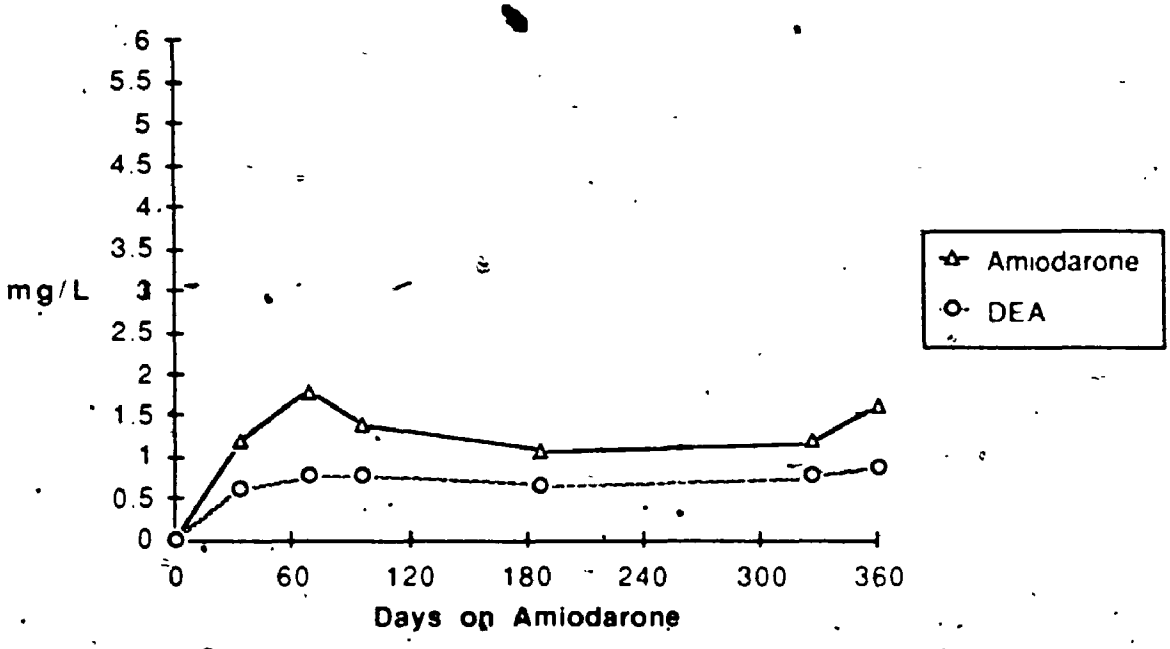
Patient 33 - Amiodarone and Desethylamiodarone Concentrations



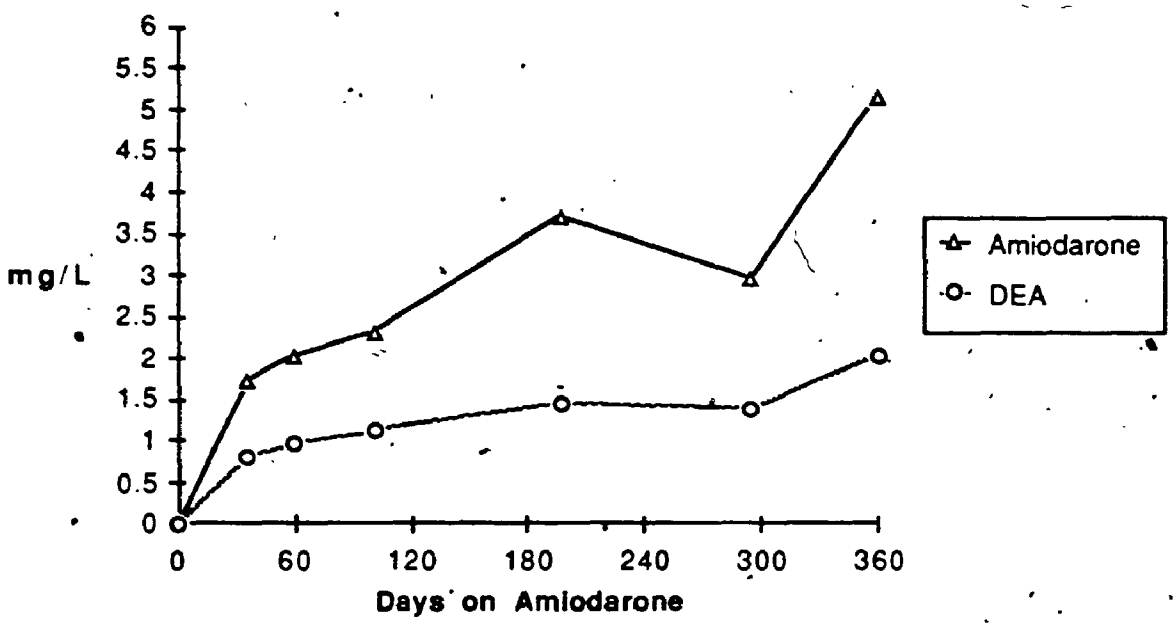
Patient 34 - Amiodarone and Desethylamiodarone Concentrations



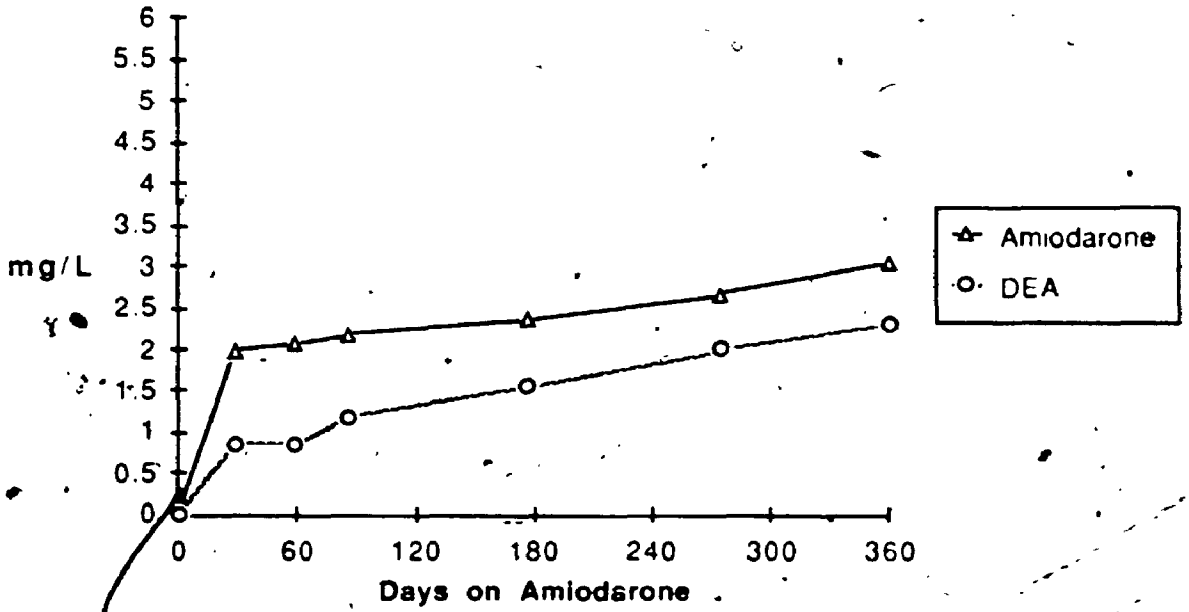
Patient 35 - Amiodarone and Desethylamiodarone Concentrations



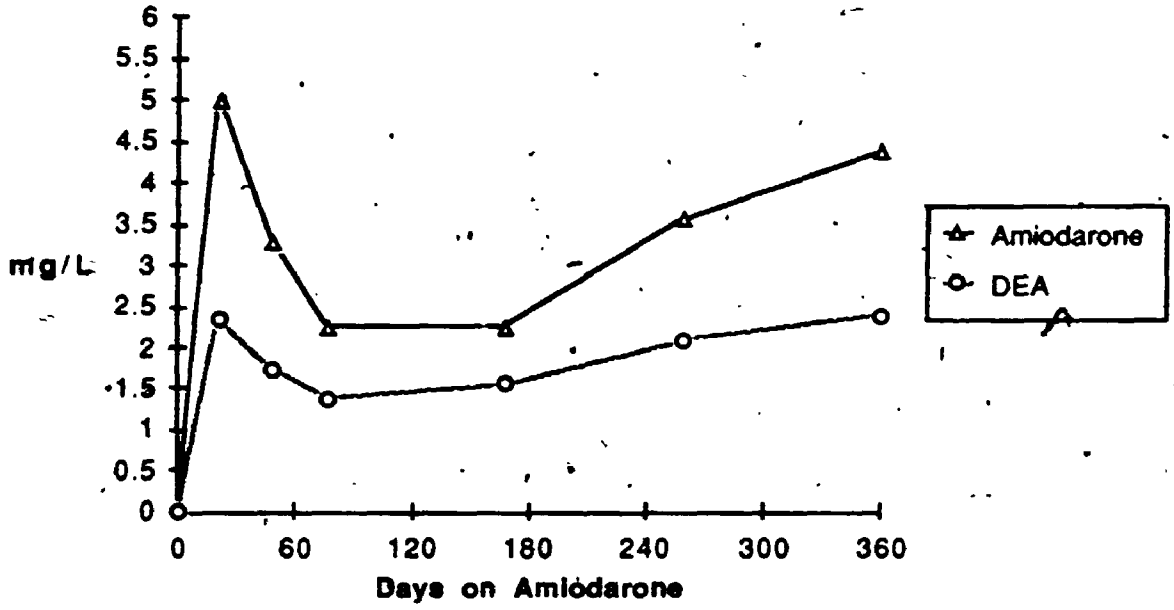
Patient 36 - Amiodarone and Desethylamiodarone Concentrations



Patient 37 - Amiodarone and Desethylamiodarone Concentrations



Patient 38- Amiodarone and Desethylamiodarone Concentrations



APPENDIX 7.

Estimation of the elimination constant from serum drug concentrations prior to steady state

Estimation of the elimination constant from serum drug concentrations prior to steady state

The concentration of a drug in blood is determined by its rate of absorption, dose size, dosing interval, time since starting dosing (number of doses), time since last dose, volume of compartments, interdepartmental rate constants and rate of elimination. When the other determining factors are held constant, the rate of elimination can be estimated from serum drug concentrations during the accumulation phase. The special case of a continuous infusion provides a simplified example. Since dosing is into the central compartment and is infinitely frequent, the terms for absorption time, dose size and dosing interval are replaced by the infusion rate constant. The general equation for the concentration of drug during an infusion into the central compartment of a general model with n compartments is as follows (Gibaldi and Perrier 1982):

$$C = \frac{k_0}{V_c}\left[\frac{\prod_{i=2}^{n} E_i}{\prod_{i=1}^{n} \lambda_i} - \sum_{l=1}^{n} \frac{\prod_{i=2}^{n}(E_i - \lambda_l)}{\lambda_l \prod_{\substack{i=1 \\ (i \neq l)}}^{n} (\lambda_i - \lambda_l)} e^{-\lambda_l t}\right] \qquad (1)$$

where:

- t = time since beginning of infusion
- C = concentration at given time (t)
- k_0 = infusion rate;
- V_c = the volume of central compartment;
- E_i = transfer constant towards central compartment;
- λ_i = disposition rate constant.

This equation demonstrates that when the infusion rate, volume of the central compartment and transfer constants are not varied, the concentration is an eponential function of time. The most common pharmacological model is for two compartments. When n=2, $E_i = k_{21}$ and equation 1 can be expanded to:

$$C = \frac{k_o}{V_c}\left[\frac{k_{21}}{\lambda_1 \cdot \lambda_2} - \left(\frac{k_{21}-\lambda_1}{\lambda_2(\lambda_1-\lambda_1)}e^{-\lambda_1 t} + \frac{k_{21}-\lambda_1}{\lambda_2(\lambda_1-\lambda_2)}e^{-\lambda_2 t}\right)\right] \quad (2)$$

$$C = \frac{k_o \quad k_{21}}{V_c \lambda_1 \cdot \lambda_2} - \frac{k_o \quad k_{21}-\lambda_1}{V_c \lambda_2(\lambda_1-\lambda_1)}e^{-\lambda_1 t} - \frac{k_o \quad k_{21}-\lambda_1}{V_c \lambda_2(\lambda_1-\lambda_2)}e^{-\lambda_2 t} \quad (3)$$

At steady state, $t = \infty$ causing the exponential terms to approach zero and the concentration C_{ss} is related to the infusion rate and the volume of the central compartment as follows:

$$C_{ss} = \frac{k_o \quad k_{21}}{V_c \lambda_1 \cdot \lambda_2} \quad (4)$$

Substitution of equation 4 into equation 3 produces:

$$C = C_{ss} - \frac{k_o \quad k_{21}-\lambda_1}{V_c \lambda_2(\lambda_1-\lambda_1)}e^{-\lambda_1 t} - \frac{k_o \quad k_{21}-\lambda_1}{V_c \lambda_2(\lambda_1-\lambda_2)}e^{-\lambda_2 t} \quad (5)$$

Because the term $\lambda_1 >> \lambda_2$ (normally the deeper compartment, 2, has the smaller disposition rate constant) at later times during infusion these negative terms multiplied by the larger t values will cause $e^{-\lambda_1 t}$ to approach zero. The relationship then simplifies to:

$$C = C_{ss} - \frac{k_o \quad k_{21}-\lambda_1}{V_c \lambda_2(\lambda_1-\lambda_2)}e^{-\lambda_2 t} \quad (6)$$

The last term in equation 6 maybe multiplied by one i.e. $\frac{k_{21}}{k_{21}} \div \frac{\lambda_1}{\lambda_1}$

$$C = C_{ss} - \frac{k_o \dfrac{k_{21}(k_{21}-\lambda_1)}{k_{21}}}{V_c \dfrac{\lambda_1 \lambda_2 (\lambda_1-\lambda_2)}{\lambda_1}} e^{-\lambda_2 t} \qquad (7)$$

Equation 4 can again be substituted in equation 7 to show the other C_{ss} term

$$C = C_{ss} - C_{ss} \cdot \frac{(k_{21}-\lambda_1)}{k_{21}} \frac{(\lambda_1)}{(\lambda_1-\lambda_2)} e^{-\lambda_2 t} \qquad (8)$$

Rearranging and taking the natural log of both sides of the equation produces a linear equation of the form y = b - mx:

$$\ln\left(\frac{C_{ss}-C}{C_{ss}}\right) = \ln\left[\frac{(k_{21}-\lambda_1)}{k_{21}} \frac{(\lambda_1)}{(\lambda_1-\lambda_2)}\right] - \lambda_2 \cdot t \qquad (9)$$

Therefore a plot of concentrations prior to steady state in the form $\ln (C_{ss} - C/C_{ss})$ vs. time should produce a final linear segment with a slope equal to the disposition rate constant for the 2nd compartment. (For $\log_{(10)}$ plots the slope is multiplied by 2.303) This disposition rate constant is approximately equal to the terminal elimination rate constant when distribution is much more rapid than elimination. Thus half-life ($T_{1/2}$) can than be calculated as:

$$T_{1/2} = \frac{0.693}{\lambda_2} \qquad (10)$$

A parallel set of equations can be developed for models up to n compartments and expanded to contain other constants for absorption rate, dose size and dosing interval. These equations would then describe multiple oral dosing. Therefore during the accumulation phase of a consistent multiple dosing regimen, a plot of concentrations sampled at identical times post dosing can be used to estimate the terminal elimination half-life.

VITA

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